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HORMONES AND VITAMINS

HORMONES
AND
VITAMINS

A Handbook for Physicians and Pharmacists

BY
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PREFACE

DURING the last ten or twenty years there have been great developments in the discovery and use of hormones and vitamins and at no period of medical history have so many new therapeutic agents been introduced in so short a time.

The purpose of this book is to provide a readily accessible compilation of chemical and clinical information on hormones and vitamins to facilitate the dispensing of them by the pharmacist and the prescribing of them by both physicians and surgeons.

In addition to this, an attempt is made to present a unified conception of hormones and vitamins to form a basis upon which to memorise principles and applications and into which new facts and subsequent developments can be incorporated.

This conception may perhaps be of some assistance to medical and pharmaceutical students as well as to practising physicians and pharmacists whose opportunities for acquiring a working knowledge of the facts and principles involved may have been limited.

In dealing individually with each hormone and vitamin, established facts are first given, but in the present state of knowledge there remains much scope for speculation and the expression of individual opinion, especially in regard to the general significance of the inter-relationship of the hormones and vitamins. All such expressions must be understood to be those of the author alone.

This opportunity is taken of expressing gratitude to the Directors of The British Drug Houses Ltd., for their permission to publish and for their generosity in allowing me to use the blocks for the half-tone illustrations and for permitting the reproduction in a modified form of the diagram on page 81. Finally I express my gratitude also to pharmaceutical and medical friends and acquaintances for their interest and encouragement.

G. A. STEPHENS.

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INTRODUCTION

THE rapidity with which new hormones and vitamins have been discovered and made available for general clinical use during the last ten to twenty years has created a state bordering upon chaos for general practitioners and for pharmacists. During no period of medical history have so many new therapeutic agents been introduced in so short a time. The position is further complicated by the fact that the use of these new substances involves new principles in therapeutics. The use of the older drugs was empirical, in that most of the drugs were entirely foreign to the body and were administered in order to bring about artificial and even violent changes as a means of inhibiting or stimulating body processes in an attempt to cure disease.

As examples of such methods the use of magnesium or sodium sulphate, of antacids and of such alkaloids as strychnine, atropine or morphine will be recalled. All such drugs act by violently disturbing physiological or pathological states and are in this sense empirical.

Administration of Hormones and Vitamins

The administration of hormones and vitamins, on the other hand, restores metabolic processes to normal when such processes have been deranged as a result of deficiency, or, alternatively, if a diseased state has been brought about by an excess of a hormone or vitamin, another hormone or vitamin (particularly the former) may be administered in an effort to restore the balance.

The correct application of such a means of treatment demands a more detailed knowledge of the nature and mechanism of physiological processes than was necessary for the treatment of disease by the administration of medicinal substances essentially foreign to the body.

A Synoptic View

The acquisition of an understanding of the principles and processes involved is not easy unless a coherent and logical

conception of the inter-relationships and modes of action of the hormones and vitamins is first obtained. Such a synoptic view of hormones and vitamins as a whole can then form a basis upon which the new body of knowledge can be built up, into which new information can be fitted, and which will simplify the memorising of a mass of otherwise apparently unrelated facts. It is the presentation of this synoptic view which is attempted in this book.

Details must in some cases be filled in by consulting other and more detailed text-books and the whole kept up to date by following recent developments as reported in the scientific journals. This, it has been suggested, can become a hobby rather than an onerous duty :—

> " Medicine is a science and at the same time an art. The art in medicine, which started as a pure art, is based on empiricism. For centuries, at first exceedingly slowly, then more rapidly, science little by little has been replacing art in medicine. This process went on steadily, but its pace increased tremendously with the general growth of scientific knowledge in the second half of the nineteenth and in the twentieth century. All sciences, with their exact methods of investigation, have contributed to the progress of medicine, but an exception in that respect has been played and will continue to be played by the experimental method as it is used in physiology, biochemistry, pharmacology, and experimental pathology, that is, in those sciences which are the foundation of medicine and surgery. Especially with the development of endocrinology, which, may be considered a truly twentieth-century science, the face of medicine is likely to change substantially during the next few decades. The physician and the surgeon of the future will become more scientifically minded, and at the bedside of the private patient and in the hospital ward they will think more physiologically than they do now. Whether you want to or not, you must follow the future development of scientific medicine. Otherwise you will be in danger of falling into a routine and of basing your judgements on obsolete theories and inexact observations, which in the meantime will have

been replaced by new and exact facts. Develop the habit of reading not only medical but also physiological journals. Make this your hobby. How much more time people spend in reading novels and detective stories, or playing bridge, for enjoyment, not realizing that scientific literature, or still more the actual performance of experiments can give an enormous amount of pleasure, hardly comparable with that derived from any other source" (*Nova Scotia Med. Bull.*, Oct. 1943, p. 219).

Unified Conception of Hormones and Vitamins

It is appropriate here to give a brief outline of this unified conception of hormones and vitamins and their physiological functions.

In the first place it is suggested that vitamins and hormones should be regarded as having originated as pathological or excretory products in plants or animals, and that plants and animals have subsequently adapted themselves and their metabolic processes to these originally abnormal metabolites, so evolving more efficient and adaptable metabolic systems.

There is growing evidence that evolution has proceeded on similar lines in regard to other originally abnormal products. Examples among plants probably include alkaloids, essential oils and anthocyanins (*Nature*, Feb. 1, 1941, p. 148). Some of these substances remain waste products of metabolism, whilst others—for example, the anthocyanins—have been adopted as integral parts of the normal plant, or, alternatively, the plant has adapted itself to utilise these originally excretion products for some specific purpose in an improved nutritional or reproductive physiology.

Some alkaloids appear to be mere excretory products of plants and to play no active part in the physiological processes of the organism, although they may serve to discourage herbivorous animals from eating the plant, and so be conducive to survival.

Essential oils may act similarly, and aromatic resins may, from the metabolic point of view, be mere waste products which incidentally protect woody tissues from attack by fungi and insects.

Among animals it has been suggested that snake venoms may have been pathological products of dental decay or buccal abscesses which became conducive to survival because they assisted snakes in overcoming organisms from which they obtain their food.

All these acquired characteristics, and many others, such as protective and attractive colouring in animals, attractive (to insects) colouring and perfume in flowers, are acknowledged to be the outward factors in natural and sexual selection, the vehicles of evolution, and the production of these in the first instance may be the result of organisms having the power of profiting from misfortune and adapting unpromising waste products for vital purposes.

Evidence of such processes from the palæontological record of evolution can of necessity only be inferential, for only the gross results of its working can survive. Comparative anatomy, histology and physiology can provide some evidence, and the physiology of fœtal development may be expected to provide a very great deal of evidence as the details of the biochemistry of fœtal growth and development are elucidated.

Eventually it may be possible to formulate a definite and comprehensive conception of the adaptation of waste or pathological products by plant and animal organisms for beneficial purposes. In this book, however, attention will be confined only to that heterogeneous group of substances included under the names hormones and vitamins.

"Conclusions", or perhaps provisional hypotheses, are drawn from facts rather freely in this book, but this is done deliberately and with a purpose—to facilitate the acquisition and retention of a diversity of facts.

PART I

HORMONES

CHAPTER I

DEFINITION

HORMONES have been described as chemical messengers, but this is an inadequate description. They should be described rather as initiators and stimulators of metabolic processes without which growth would cease and the body cease to " live ", for not only do they deliver the " message ", but also take part in the subsequent activity. Most of the hormones appear to be enzymatic in nature—that is, they activate chemical processes without themselves being appreciably changed as a result of the reaction. The true enzymes of the body are for the most part not secreted by special organs, such as the endocrine (internally secreting) glands, as are the hormones, but are secreted by various tissues whose characteristic structure is adapted to some quite different function.

For example, pepsin is secreted by the stomach, the primary function of which may be said to be the reception and mechanical digestion of food. Pepsin secretion is a secondary function of the stomach. The secretion of hormones, on the other hand, appears to be the sole function of most of the endocrine glands, such as the pituitary, the thyroid and the suprarenals. Some of the endocrine glands combine some other function with their secretory activity. The ovaries and testes produce gametes (ova and spermatozoa respectively) as well as their characteristic hormones. The pancreas is unique, in that it is established that it produces both an internal secretion (insulin) and an external secretion (pancreatic juice, containing a mixture of enzymes). Other glands, including the prostate, have an external secretion, and may also be endocrine organs.

Hormones initiate and sustain physiological action, some by means comparable with that of enzymes, and others by some means as yet unelucidated. Some hormones act

directly, as does the ovarian hormone in stimulating proliferation of uterine and mammary tissue, whilst others, particularly those of the anterior lobe of the pituitary, act indirectly by stimulating the secretory activity of other glands.

Like the true enzymes, hormones are active in extremely minute amounts. They are probably not changed in consequence of exerting their specific activity, but are inactivated later, partially or completely, when their specific function has been carried out.

Isolation of Pure Substances

Partly because of the minute amounts produced by the body, considerable difficulty was at first encountered in trying to isolate the pure hormones. Thus gland extracts and dry gland substances came to be used empirically in the hope of producing therapeutic effects. With most glands this inevitably led to disappointment, for not only are the hormones generally less active (or even quite inactive) when given by mouth, but the hormone content of such preparations is generally negligible. Thyroid gland is an exception, for not only does it secrete the hormone but also stores it, and, further, the hormone is not much affected by the gastric juices.

The ovaries are typical in this respect, however. Ovarian hormone is appreciably less active when given by mouth than when injected or secreted in the body, and the gland does not store its hormone. Indeed, 1 ton of fresh cow's ovaries will yield only about 6 milligrams of hormone (œstradiol) (*Journal Amer. Med. Assoc.*, Feb. 8, 1941, p. 502), an amount which is commonly given in one dose. The sale of ovarian " extracts " (dry gland substance) is illegal in the U.S.A. (*Journ. Amer. Med. Assoc.*, Feb. 3, 1940, p. 415), and in Canada the following phrase must appear on labels of ' any preparation which consists wholly or in part of sex-gland tissue or extracts thereof : . . . "This preparation does not possess sex hormone activity ".'

Groups of Hormones

Chemically the hormones are not so diverse as the vitamins. They can be grouped into two main classes : the " nitrogenous " hormones, which are amino-acid derivatives (adrenaline and thyroxine), polypeptides or proteins (insulin, the gonado-

tropins and posterior pituitary hormones), and the steroid hormones (sex hormones and suprarenal cortex hormones). There is a third group, the existence of which is doubtful, but which, if they are shown to exist, will possibly prove to be polypeptides or proteins. In this group are the hypothetical hormones of the thymus, prostate and pineal glands.

In a somewhat wider sense, many substances normally thought of as vitamins are in certain circumstances to be classed among the hormones. Many vitamins (for animals) are hormones as far as plants are concerned, for they are elaborated by the plants themselves, and the plants utilise them in metabolic processes of growth and development. Most of the members of the vitamin B group are examples of this. So far as is known, these substances are hormones for one or more plants but vitamins for animals, who do not synthesise them. The universal applicability of this statement has been questioned, but it has been vindicated by comparatively recent findings, which have shown that these substances are elaborated by bacteria normally present in the intestines of many animals which absorb them from this source, and are consequently independent of dietary sources of these vitamins. Efficient intestinal antiseptics, such as succinylsulphathiazole, kill such intestinal bacteria and, until the bacteria can be re-established, the animal becomes dependent on dietary sources.

Ascorbic acid is a hormone for all animals except the primates and guinea-pigs, and even the primates can synthesise ascorbic acid during infancy. Human infants, for example, can synthesise it for about the first three months of life, after which they lose this power, and the hormone then becomes a vitamin for them.

Thus, before a definite statement can be made as to whether a given substance is a hormone or a vitamin, it is necessary to know if the statement is to be made from the standpoint of plants or of animals, and of what particular species of animal.

There is, therefore, no clear-cut differentiation between hormones and vitamins. In this book, however, the distinction is made from the standpoint of humans.

NITROGENOUS HORMONES

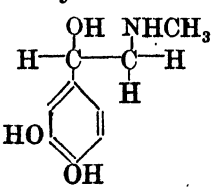
CHAPTER II

ADRENALINE

THE blood-pressure-raising activity of the medulla of the suprarenal glands was discovered in 1894 by Schäfer, but it was not until 1901 that Takamine isolated the hormone, which he called adrenaline. Takamine appears to have been assisted by the earlier work of Abel, and possibly by that of von Fürth, who named the hormone epinephrine before it had been isolated. Aldrich also isolated the hormone, and published his report at about the same time as Takamine. The chemical structure of adrenaline was established as a result of the combined efforts of at least eight investigators, and was confirmed as a result of synthesis by Stolz (German and English patents, 1903, and a paper in 1904). The synthetic substance was a racemic mixture, but Flächer resolved this mixture, producing the lævorotatory compound identical with the natural hormone in 1908.

Chemical Nature of Adrenaline

Chemically adrenaline is probably the simplest of all hormones. Its structure is indicated by the chemical name *l*-3 : 4-dihydroxy-α-phenyl-β-methylamino-ethanol, and the formula :



It is related to the amino-acid tyrosine, which may be a precursor of this hormone, as it is of thyroxine.

The official salt of adrenaline for medicinal use is the hydrochloride, and this, like the hormone itself and the other salts, is readily oxidised and inactivated. In the suprarenal

gland adrenaline appears to be protected to some extent by ascorbic acid (vitamin C, *q.v.*). Adrenaline hydrochloride is therefore dispensed in slightly acid solution. It should be protected from strong light, and solutions should be sterilised by heating in a closed container at 80° for 1 hour. Solutions should be stored in well-filled and well-closed containers, protected from light and in a cool place.

Adrenaline base is only slightly soluble in water and is almost insoluble in the common organic solvents. It is a reducing agent, and readily reduces Fehling's solution, particularly in the presence of minute traces of iron.

Methods of Administration

When administered by the mouth, adrenaline appears to exert some local effect, but it is rapidly inactivated, and no general systemic effects are produced. The local effect has been used as a means of controlling gastric hæmorrhage. Generally adrenaline is administered by intramuscular or subcutaneous injection. By this route it is effective in controlling allergic symptoms, serum reactions and asthmatic paroxysms, as well as in relieving the hypoglycæmia resulting from an overdose of insulin.

Potency of Adrenaline

The potency of adrenaline is not expressed in terms of units. It is normally prepared as the virtually pure substance, and biological units are therefore unnecessary. Although it is a simple chemical substance, there is no simple chemical means of estimating adrenaline in preparations containing even small amounts of other substances. The estimation can readily be done, however, by means of a physiological test of its blood-pressure-raising properties in a cat or dog.

The British Pharmacopœia 1932 gives a chemical test for the identity of adrenaline. An emerald colour is given by adrenaline in neutral or slightly acid solution with a dilute solution of ferric chloride. Solution of sodium bicarbonate added to this changes the colour to blue and then to red.

The pharmacopœial tests for purity are physical; melting point (205° to 212°, when the increase in the temperature is 10° per minute), specific rotation (in 4 per cent. w/v solution

in N/1 hydrochloric acid), 50° to 53° and not more than 0·01 per cent. of ash after incineration.

Two colorimetic methods of estimating adrenaline are available, and details of these will be found in Allport's " Colorimetric Analysis," p. 326 (Chapman and Hall), 1945.

Physiological Action of Adrenaline

The medulla of the suprarenal gland may be looked upon as a large and specially developed nerve-ending in the sympathetic nervous system. Under special conditions of emotion the gland secretes adrenaline, which is indistinguishable from the normal chemical transmitter substance of the rest of the sympathetic nervous system. The effects of adrenaline are the same as those of stimulation of the sympathetic system—that is, the production of the bodily symptoms of fear or rage. For this reason the suprarenal glands have been called the glands of " fight or flight ". The secretion of adrenaline is largely under emotional control, and fear or rage causes an outpouring of adrenaline. The body is thereby prepared for " fight or flight ". The heart is accelerated and the force of its beat increased. At the same time, the blood pressure is raised, and the increased pressure distends the aortic arch and the carotid sinus. Nervous impulses from the arch and the sinus affect the cardiac centre, and the heart is slowed once more. The surface capillaries contract, producing blanching and paleness. In consequence of this, the blood supply of viscera and deep muscular tissue is increased. The arrectores pili and other smooth muscles of the skin are stimulated, so that the hair may " stand on end ". The excitability of skeletal muscle is increased and fatigue is counteracted, partly as a result of increased conversion of liver glycogen into glucose. The pupils are dilated and the eyes may protrude slightly.

The sum of all these effects is that the body is keyed up to the level of nervous and muscular energy and preparedness most suitable for effective flight from danger or for putting up an effective fight if the will decides that the danger is to be faced and overcome. Further, if the latter course is adopted and injury results, then hæmorrhage will be limited as a result of the constriction of the peripheral blood vessels.

The action of adrenaline is antagonistic to that of insulin in

that it raises the blood-sugar level. This it does in two ways : directly, by stimulating the mobilisation of liver glycogen and its conversion into glucose, and indirectly, by promoting the conversion of lactic acid to glycogen in the liver.

The Chemistry of Adrenaline Action

The chemistry of the action of adrenaline is unknown, but its outstanding characteristic, ready oxidisability, seems to suggest that it may be an essential factor in some reduction process. This hypothesis appears to be supported in some measure by the fact that a mechanism has been described whereby ascorbic acid (vitamin C) reacts with oxidised adrenaline in the suprarenal medulla and reduces it once more to adrenaline (*Biochem. Journ.*, 1935, **29**, 998).

Clinical Uses of Adrenaline

The pressor or vasoconstrictor property of adrenaline is the one most commonly made use of clinically, as a means of controlling hæmorrhage, and as a means of prolonging the effect of a local anæsthetic and inhibiting its spread and dispersal over an unnecessarily large area.

Adrenaline in solution (1 in 1000) is applied locally as a hæmostatic, and has been administered orally in doses of ½ to 1 fl. oz. for the control of hæmatemesis. As a means of inhibiting the dispersal of local anæsthetics, 1 in 1000 solution of adrenaline is added to the anæsthetic solution in various proportions from 1 to 1 to 1 to 12. In dentistry, the concentration of adrenaline is usually 1 in 5000 to 1 in 50,000 of the anæsthetic solution.

To relax the bronchiolar muscles and to relieve asthmatic paroxysms, 5 to 10 minims of 1 in 1000 solutions of adrenaline is given intramuscularly or subcutaneously. For the relief of anaphylactic shock, serum reactions and hypoglycæmia, ½ to 1 c.c. is usually required. A rather smaller dose (0·2 to 0·5 c.c.) is generally effective in relieving urticarial reaction to a protein.

In acute cardiac failure or collapse under an anæsthetic, 1 to 1·5 c.c. may have to be injected intracardially.

It will be noted that the official dose (0·12 to 0·5 ml.) of solution of adrenaline hydrochloride is commonly exceeded.

Contra-indications to Adrenaline and Effects of Overdosage

Adrenaline should be administered cautiously and in small doses to patients with cardiac disease, hyperthyroidism or in asthma after morphine has been given.

The effects of overdosage are the production of the symptoms described under " Physiological Action " to an exaggerated degree.

Supplementary Note

The important facts of the action are accounted for in the foregoing, but comparatively recent findings seem to indicate that the subject may be considerably more complex than has so far been suggested.

Adrenaline is certainly given off by adrenergic nerves, but it appears to be subjected to modifications before exerting its characteristic effects. According to one theory, adrenaline is altered to form sympathin. Sympathin, it is suggested, exists in two forms : sympathin E and sympathin I. The first is responsible for the excitatory effects attributed to adrenaline, and the second the inhibitory effects.

According to an alternative theory, adrenaline itself exerts an excitatory effect, and the inhibitory effects are produced by " adrenoxine," a substance produced by partial oxidation of adrenaline.

These theories are reviewed and references are given in Heilbrunn's " Outline of General Physiology " (W. B. Saunders Co., 1943).

CHAPTER III

THYROXINE AND IODOTHYROGLOBULIN

NO definite knowledge of the function of the thyroid gland was established until Sir William Gull correlated thyroid atrophy with a syndrome in middle-aged women which consists of loss of hair, thickening and dryness of the skin and marked loss of mental and physical vigour. In 1878 William Ord called the condition myxœdema, because he thought that the thickening of the subcutaneous tissues was due to the formation of mucin. Gull's observation was confirmed by the results of thyroidectomy for the treatment of goitre in man (1882 and 1883).

Various species of animals were subjected to thyroidectomy, and in dogs and cats this resulted in the onset of tetany. Some confusion arose in consequence of this until Gley, in 1891, rediscovered the external parathyroid glands, and showed that the tetany was not a symptom produced by removal of the thyroid, but by the parathyroidectomy accidentally complicating the thyroidectomy.

In the same year Murray showed that a glycerin extract of sheep's thyroid was curative in a case of "Gull's disease" (myxœdema). The patient was in an advanced stage of disease when treatment was started, but was restored and maintained in health until she died in 1919 at the age of seventy-four years.

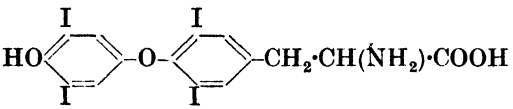
In 1892 several investigators showed that thyroid substance, either fresh, dried or cooked, was equally efficacious when given by mouth.

As early as 1820 Coindet had shown that iodine was effective for the treatment of goitre, and many attempts were subsequently made to demonstrate that thyroid contained iodine. This was accomplished by Baumann in 1895. The next advance was the observation of Oswald in 1899 that the iodine was contained in the colloid of the gland and that the colloid was a globulin for which he introduced the terms "thyroglobulin" and "iodothyroglobulin".

In 1916 Kendall isolated a substance containing 65 per cent. of iodine, and Harington determined its empirical formula ($C_{15}H_{11}O_4NI_4$) as well as its structure, proving it to be " 3 : 5 : 3′ : 5′-tetraiodothyronine ", a substance which he and Barger synthesised in 1926. This compound, generally known as thyroxine, is commonly regarded as the hormone of the thyroid gland. The true hormone, however, is possibly Oswald's iodothyroglobulin, of which thyroxine is a prosthetic group. Further, thyroxine does not account for the whole of the iodine of the iodothyroglobulin molecule. It may be that di-iodotyrosine is also a component of the hormone. Harington has shown that probably 40 per cent. of normal thyroid iodine is in the form of thyroxine and the remaining 60 per cent. di-iodotyrosine.

Chemistry of Thyroxine and Iodothyroglobulin.

Thyroxine is β-[3 : 5-di-iodo-4-(3′ : 5′-di-iodo-4′-hydroxyphenoxy)phenyl]-α-aminopropionic acid, the structure of which is represented by the formula



Thyroxine is almost certainly synthesised in the thyroid gland from 3 : 5-di-iodo-4-hydroxyphenylaminopropionic acid, more commonly known as di-iodotyrosine, two molecules of which combine to form one molecule of thyroxine and one or more unidentified substances probably including alanine.

It seems certain that di-iodotyrosine does not share any of the physiological properties of thyroxine, and for this reason it is frequently regarded as being physiologically inert. This is probably an erroneous assumption, however, and there seems to be some evidence that di-iodotyrosine has an antithyroid action and may be of value in lowering the metabolic rate in cases of hyperthyroidism in spite of the fact that it is almost certainly a precursor of thyroxine.

Both thyroxine and di-iodotyrosine have been isolated from

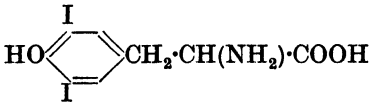
the products of the hydrolysis of iodothyroglobulin, and it is probable that thyroxine is an active prosthetic group of the complete molecule of the hormone. Di-iodotyrosine may also prove to be an active group in the molecule.

Iodine is rapidly fixed in the thyroid gland after ingestion, but there is a delay of some hours before the administration of iodine brings about any increase in thyroxine synthesis and produces any increase in the metabolic rate. This rapid fixing of iodine in the body is carried out by some means at present not understood. It may be, however, that the iodine combines immediately with tyrosine, either in the free state or as a component of the thyroglobulin as distinct from the *iodo*thyroglobulin molecule. It seems to be reasonable to suggest that some delay in the incorporation of free thyroxine into the thyroglobulin molecule after administration may account for the delay in the appearance of therapeutic effects. This suggestion is entirely hypothetical, however, and there is no objective evidence to support it.

Thyroxine itself is insoluble in water, so that for medicinal use thyroxine-sodium is employed and is official in the British Pharmacopœia. Thyroxine is soluble in alkaline solutions, but is unstable, so that solutions of thyroxine-sodium should be neutral. It is reasonably stable to heat, but should be stored in well-closed containers. Dried thyroid should also be protected from moisture and from heat, although it is probably stable to dry heat.

The most recent information available indicates that the thyroid gland concentrates iodide within itself and converts it into free iodine by virtue of an oxidase system. The iodine combines with tyrosine contained in the thyroglobulin molecule, and then an intramolecular reaction takes place, forming thyroxine. This last reaction may be activated by an enzyme system, or free iodine may serve as an oxidant, so that the reaction may take place in the absence of an enzyme. Indeed, the whole reaction takes place *in vitro* in the iodination of casein to form thyroxine in the absence of the enzyme. It is probable that the nature of the protein is important. For example, silk fibroin is rich in tyrosine, but thyroxine is not formed when it is iodinated (*Science Progress*, July 1946, p. 586).

Tyrosine is 4-hydroxy-α-aminopropionic acid, so that the 3 : 5-di-iodo-compound has the following structure :—



and its relationship to thyroxine will readily be seen.

Di-iodotyrosine appears to be reasonably stable, and is normally administered orally in the form of tablets.

Standardisation of Thyroid and Estimation of Thyroxine

Dried thyroid gland substance is standardised in terms of its content of iodine in combination as thyroxine. Thyroxine-sodium is estimated in terms of its iodine content, 1 part of iodine being equivalent to 1·57 parts of thyroxine-sodium.

The method of estimation for both thyroid and thyroxine is described in the first Addendum, 1936, to the British Pharmacopœia, 1932.

Because of the simplicity and accuracy of this chemical method of assay, biological units and methods of assay are unnecessary.

Physiological Action of Thyroid

The action of thyroid is that of stimulating metabolism, particularly the oxidation of carbohydrates, fats and the non-nitrogenous parts of proteins. The metabolic rate is increased, and the amount of increase is indicated by the increase in oxygen intake.

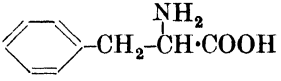
This relationship between the thyroid gland and metabolism is of interest, and possibly of significance in relation to the embryological origin of the gland. Early in the life of the embryo (fourth week) the buccal and pharyngeal parts of the tongue are appearing as elevations on the floor of the primitive pharynx. The hypoblast in the furrow between these two parts of the tongue thickens, forming a bud which grows downwards and backwards and soon bifurcates. It then re-divides to form a network of acini, and becomes the isthmus of the thyroid gland. The lateral lobes of the thyroid are formed in the recesses of the fourth pharyngeal cleft. These pockets lose their connection with the hypoblastic lining of the pharynx and

become isolated buds—which divide and re-divide to form a collection of isolated acini. As they grow, these lateral lobes come in contact with the median lobe (isthmus), forming the thyroid gland as seen in the adult. It seems likely that the isthmus of the thyroid was once an " exocrine " gland which poured its secretion into the mouth. (Keith, " Human Embryology and Morphology ", 1902).

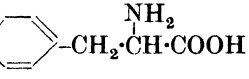
It is interesting to note that proteins, or more specifically certain of their constituent amino-acids, have a " specific dynamic effect " in increasing metabolism. This effect appears to be associated with the formation of glucose from the amino-acids, the most effective of which are possible precursors of tyrosine, the amino-acid radicle of both di-iodotyrosine and thyroxine. The most active amino-acids in this respect are, in order of increasing activity

Alanine $CH_3 \cdot \underset{\displaystyle NH_2}{\underset{|}{C}}H \cdot COOH$

Glycine $\underset{\displaystyle NH_2}{\underset{|}{C}}H_2 \cdot COOH$

Phenylalanine 

all of which should be compared with

Tyrosine 

an intermediate in the synthesis of thyroxine.

It appears, therefore, that there is some reason to suppose that in the course of evolution the thyroid gland and its hormone have changed their rôle of secondary factors in metabolism to primary factors, the hormone now having a generalised function on practically all body-cells and metabolic processes.

As has been indicated previously, it has for some time been considered that the hormone of the thyroid gland is a complex consisting of a globin to which thyroxine and di-iodotyrosine are attached as prosthetic groups. This view appears to have been that of Harington (*Brit. Med. Journ.*, Dec. 19, 1936, p. 1269), but he now seems to consider that the actual hormone of the thyroid gland is not iodothyroglobulin, but thyroxine (*Nature*, July 22, 1944, p. 122). May it not be that iodo-

thyroglobulin is the storage form of the hormone (it is the principal component of the thyroid colloid), that thyroxine is the mobile transport form (analogous to unconjugated vitamins), and that thyroxine combines with a protein-carrier at the site of its action? This thyroxine-protein complex may be the active form of the hormone, enzymatic in nature and, again, analogous to the vitamin-protein complexes such as the dehydrogenases, which are the metabolically active forms of most of the vitamins.

This view appears to be reasonable, whatever the precise mechanism of the thyroid action as an accelerator of metabolism proves to be, since it is at least compatible with a conception of thyroxine entering body-cells and these combining with a protein (globin) and assuming its active form in a manner precisely analogous to the entry and activation of the vitamins of the B group; further, it must be recalled in this connection that the "vitamin-B" enzymes (holodehydrogenases) are perhaps the most important factors in maintaining the normal metabolic precesses.

Clinical Uses of Thyroid Gland Substance and Thyroxine

There are two main conditions of marked thyroid deficiency in which the gland or its "active principle", thyroxine, is indicated: cretinism and myxœdema. Cretinism may result from failure of development of the thyroid during embryonic life or atrophy of the thyroid during fœtal life or early infancy. Physical and mental development is minimal in cretins, who are thus dwarfs and idiots. They are malformed and have protuberant abdomens, thick coarse hair, dry and pale skin, gaping mouth and protuberant tongue. The hands and feet are spade-like and the lips are thick. High blood cholesterol levels usually occur, and may be used as an additional diagnostic test. As soon as cretinism is diagnosed in an infant, thyroid should be administered; with suitable doses, relatively normal development can be achieved, particularly if diagnosis is made and treatment begun sufficiently early. The symptoms may be somewhat indefinite during the first few weeks of life, so that blood cholesterol estimations are particularly valuable. Treatment is not curative, but substitutional, so that it must be continued throughout life.

Myxœdema is virtually the same as cretinism, with the difference that its onset occurs much later in life and is most common in middle-aged women. Like cretinism, myxœdema is the result of atrophy of the thyroid gland. It is insidious, and appears first as a diminution in physical and mental function. In its early stages, therefore, it may be mistaken for the normal dimming of the faculties through age. Estimations of blood cholesterol may provide valuable diagnostic evidence before any of the symptoms have become sufficiently pronounced to form a certain basis for diagnosis. Untreated cases of myxœdema develop marked reduction in basal metabolic rate, slowing of the pulse rate and low body temperature. The hands and face become puffy and swollen, not from mucin-formation, as the name myxœdema suggests, but possibly from hypertrophy of subcutaneous connective tissue. The skin also becomes thickened and the hair tends to fall out.

As in cretinism, so in myxœdema, the administration of thyroid is specific. Early diagnosis and treatment are advisable, as in cretinism, but the results of delay are possibly not so disastrous.

Deafness may occur in myxœdema, due to swelling of the tympanic membrane. This has suggested the use of thyroxine by intratympanic injection for the treatment of certain types of deafness.

The most common use of thyroid is for the treatment of obesity. In certain selected cases and in combination with other forms of treatment it may perhaps be of some value. Close observation of the patient is essential, however, and the diet must be carefully chosen and controlled. Particular care should be taken to ensure that the diet contains adequate amounts of vitamin A and the vitamins of the B group. The patient should be carefully watched for the appearance of symptoms attributable to over-dosage, cardiac and nervous symptoms, exhaustion and glycosuria.

Thyroid appears to have an appreciable effect on the ovaries, and it is frequently of use in amenorrhœa, especially as collateral treatment in this condition with ovarian hormone.

It may occasionally be of value in certain skin diseases, particularly psoriasis and ichthyosis and in rheumatoid arthritis.

Hyperthyroid states, exophthalmic goitre and toxic adenoma are considered in the supplementary note on antithyroid substances (page 29).

Dosage and Administration of Thyroid and Thyroxine

Unlike many of the endocrine glands, the thyroid stores a considerable amount of its hormone in normal animals. In addition, the hormone is resistant to the action of the digestive enzymes. Thus the administration of dried-gland substance constitutes effective treatment.

Thyroxine is also active orally and is given by this route. It is also suitable for parenteral administration, and when it is essential to give the hormone by injection, thyroxine is employed.

Dosage of thyroid is of necessity variable from patient to patient and in one patient under varying conditions of activity of the patient's own thyroid and of general body metabolic activity. The following doses in the various indications may be taken as averages, to be varied in accordance with the response of the patient.

Cretinism

Dried thyroid substance should be given orally.

6 months to 1½ years . . .	½ to 1½ grains daily
1½ years to 8 years . . .	1 to 3 grains daily
8 years to adolescence . .	1½ to 3 grains daily

(*Prescriber*, May 1944)

Myxœdema

One grain daily, gradually increased until the symptoms disappear. The average optimum dose is 3 grains daily, but some patients may need much larger doses. Satisfactory control of dosage is generally achieved if the pulse rate is watched and not allowed to exceed 80 per minute.

Obesity

This is a popular indication for thyroid, but cases should be carefully selected and the patient closely observed for signs of excessive dosage, as previously mentioned (page 27). 1 to 2 grains may be given daily, increasing or decreasing this dose in accordance with clinical findings.

Amenorrhœa

1 to 2 grains may be given daily collaterally with an œstrogen. The thyroid may be given continuously, but the œstrogen should only be given during alternate fortnights.

Psoriasis, ichthyosis and rheumatoid arthritis

½ to 1 grain may be given daily as a general metabolic stimulant in these and similar conditions.

Whenever thyroid is given over long periods it should be remembered that it may have some cumulative effect. To obviate any adverse effects from this, treatment may be omitted every fourth week. It is also important to note that thyroid appears to deplete the liver of glycogen and to impair its glycogen-storing capacity. It may be desirable, therefore, to give extra carbohydrate to some patients during treatment, and specially close observation of patients being treated for obesity is essential.

SUPPLEMENTARY NOTE

Hyperthyroid States and Antithyroid Substances

The nomenclature of abnormal states of thyroid function and of abnormal thyroid histology is confusing unless the precise meaning of the terms is clearly understood, and it seems to be necessary to define the terms involved with a considerable degree of precision if abnormal thyroid function and the treatment of such abnormalities is to be understood.

The normal thyroid tissue consists of vesicles containing colloid and lined with a single layer of low cuboidal or flattened epithelium. Abnormal states of the thyroid are described by the following terms :—

Hyperplasia.—The state in which the normal epithelial cells are replaced with high columnar cells and the vesicles contain little or no colloid. The epithelium is plicated and duplicated, and its cells show active mitotic division. Briefly, hyperplasia signifies over-development as distinct from overgrowth or enlargement (hypertrophy), and it is indicative of hyperactivity.

Hyperfunction.—Applied to a hyperplastic gland producing excessive amounts of thyroid hormone (generally)

or to a hypertrophied but hypoactive gland producing excessive amounts of hormone by reason of its bulk and multiplicity of hypofunctional hormone-producing elements (rare).

Hypoplasia.—Subnormally developed thyroid, generally hypertrophied (enlarged), and producing inadequate amounts of hormone, as in cretinism and myxœdema.

Thyrotoxicosis (Exophthalmic Goitre or Graves' Disease).—The condition which results in excessive thyroid secretion or abnormal and toxic thyroid secretion from a generally hyperplastic and perhaps hypertrophic gland.

Toxic Adenoma (Toxic Nodular Goitre).—A condition in which islets (nodules) of hyperplastic and hyperfunctional tissue appear in a normal or hypoplastic gland, and characterised by symptoms of thyrotoxicosis.

In the hyperthyroid states, chiefly thyrotoxicosis and toxic adenoma, the whole gland (in thyrotoxicosis) or parts of it (in toxic adenoma) are hyperplastic and hyperfunctional and the hormonal output of the gland is excessive. There is also a school of thought which suggests that the secretion of the gland is qualitatively abnormal and excessively toxic.

The cause of hyperthyroid states is unknown, and until comparatively recently the only effective method of treatment was partial or complete removal of the thyroid gland. Partial thyroidectomy relieves the condition, but when the operation was first performed tetany was a frequent sequel. This disadvantage was overcome when the nature and importance of the parathyroid glands were realised and care taken not to remove them with the thyroid tissue. Two disadvantages remain, however. It is not always an easy matter to decide just how much gland tissue should be removed to reduce the patient's metabolic rate to normal and, even if this is successfully accomplished, the hyperfunction of the gland may prove to be progressive, and other partial thyroidectomies may be necessary subsequently.

Two alternatives remain : total thyroidectomy with subsequent maintenance of the patient on thyroid gland given orally for the remainder of his or her life, or the use of an antithyroid substance in an attempt either to control thyroid activity

indefinitely or to control it until a remission may render further treatment unnecessary.

It has been shown recently that the body normally possesses its own natural antithyroid substance, paraxanthine (1 : 7-dimethylxanthine). (*Nature*, June 26, 1944; *Lancet*, Aug. 14, 1943, p. 197). There is an optimum degree of concentration of paraxanthine. If this concentration is doubled, the antithyroid effect is not exerted. It is not known how this substance acts, nor whether it is the principal factor controlling thyroid activity. Possibly paraxanthine acts in the same or a similar manner to the group of antithyroid substances which have been exhaustively investigated recently and of which thiouracil is a typical example, and one which has been commonly used. It has been suggested that iodination of the thyroid hormone depends in part on a cytochrome or a peroxidase enzyme system and that thiouracil and certain other organic sulphur compounds inhibit this enzyme system (*Journ. Clin. Endocrinol.*, May 1944, p. 213).

It has been found that a number of organic sulphur compounds, including sulphaguanidine, succinylsulphathiazole, phenylthiourea, thiourea and thiouracil, are goitrigenic to the lower mammals. That is, when given to normal animals they inhibit the formation of thyroid hormone and there is a compensatory hypertrophy of the gland. A similar effect is produced in man, but considerably larger doses are required before the glandular hypertrophy is produced.

The inhibition of hormone formation is produced on relatively small doses, however, and thiouracil in particular has been found to be of special value for the treatment of hyperthyroid states. When given in doses (divided) of 0·4 to 0·6 grm. daily, inhibition of the synthesis of thyroid hormone begins at once. A few patients have reported almost immediate improvement in their subjective condition, but clinical improvement is not generally detectable until about ten to twenty days after treatment is started. This is particularly true in nodular goitre (toxic adenoma), in which there is a considerable store of hormone in the gland. Similarly, patients with Graves' disease (thyrotoxicosis, diffuse exophthalmic goitre) who have received iodine may have a considerable store of thyroid hormone. When this store becomes depleted, and in untreated

patients with Graves' disease, the dosage of thiouracil is appreciably less. The dose is variable, but is generally between 0·05 and 0·2 grm. daily. The precise amount must be determined for each patient and adjusted to such a level that the hyperthyroid symptoms are eliminated but symptoms of hypothyroidism do not appear.

Throughout the treatment the patient should be kept under observation, especially during the early stages, for recurring hyperthyroidism (an increase in pulse rate is an early indication of this) and for toxic reactions to thiouracil. Such reactions include cutaneous eruption, pyrexia, arthralgia, leucopenia and jaundice. An examination of the blood should be made at intervals in order to detect leucopenia, and it is an advantage to estimate the blood-cholesterol level at the same time. A low level of cholesterol is an indication of hyperthyroidism and inadequate dosage of thiouracil. The normal blood cholesterol level is 140 to 180 mg. per 100 c.c. of whole blood.

It is not yet certain whether treatment with thiouracil is curative and can eventually be discontinued, but Astwood has reported (*Journ. Clin. Endocrinol.*, June 1944, p. 229) that nine patients have apparently been cured and that the administration of thiouracil has been discontinued after several months of treatment and there has been no return of symptoms.

Thiouracil may also be used to prepare patients for thyroidectomy in those cases in which the response to iodine is unsatisfactory. It must be borne in mind, however, that when thiouracil is used for this purpose, the gland may become soft, not easily palpable and possibly more vascular than after preparation with iodine.

If it is decided to change the treatment of a patient receiving iodine and to give thiouracil instead, the patient should be warned to expect an exacerbation of hyperthyroid symptoms, and it may be three or four weeks before the condition can be brought under control again. The longer the treatment with iodine has been continued, the longer is the period of renewed hyperthyroidism likely to continue.

All the antithyroid substances so far employed have some degree of toxicity and various alkyl derivatives of thiouracil have been tried. Of these, propyl-thiouracil appears to be

the most potent, and is less toxic than thiouracil itself. Methylthiouracil, however, is appreciably less toxic than thiouracil and slightly more active. It is replacing thiouracil. The initial dosage is the same as for thiouracil, but it is generally found that the thyrotoxicosis is brought under control more quickly and subsequent dosage is rather lower than when thiouracil is employed.

CHAPTER IV

PARATHYROID HORMONE

HYPERSECRETION of parathyroid hormone produces osteitis fibrosa cystica, the first recorded case of which is said to be that described in 1700 by Courtial. The condition was not associated with the parathyroid glands until 1906, however, when Erdheim first drew attention to enlargement of glands in cases of alleged osteomalacia which probably included some cases of osteitis fibrosa cystica. In this last condition the parathyroid enlargement is probably primary, but only a secondary symptom in osteomalacia.

Tetany was recognised as a parathyroid deficiency state during 1908 and 1909, although post-thyroidectomy tetany had been reported in 1880, 1884 and 1885.

The external pair of parathyroid glands was discovered in 1891, and an early theory of their function was that of an antitoxic activity. The work of Berman and Hanson in 1925 suggested a relationship between parathyroid function and calcium metabolism, a relationship which was eventually established in 1927 by Collip.

Chemistry of Parathyroid Hormone

The hormone of the parathyroid glands is a protein, rapidly inactivated by trypsin and pepsin. It has certain resemblances to insulin in both physical and chemical properties. It contains sulphur and possibly iron, but no phosphorus. Dodds *et al.* found that their picrate method for the preparation of insulin was also applicable to the preparation of the parathyroid hormone.

Parathyroid hormone is water-soluble, and an active solution of it is prepared by acid hydrolysis of ox parathyroids. The hormone was for long thought to be active when administered orally, but it now seems to be probable that it is inactivated by digestive enzymes (*Canad. Med. Assoc. Journ.*, 1931, **24**, 646).

Unit and Potency of Parathyroid Hormone

There is no international standard or unit for parathyroid hormone, and an arbitrary biological unit is employed. The unit most commonly used is that of the U.S.P. XII. : " one-hundredth of the amount required to raise the calcium level of 100 c.c. of the blood serum of normal dogs 0·001 gm. within from sixteen to eighteen hours after administration ".

Physiological Action of Parathyroid Hormone

Nothing is known of the chemistry of the mode of action of the parathyroid hormone. It has been noted, however, that the hormone activates kidney phosphatase *in vitro*, but this effect is not specific, for egg albumin and serum albumin produce a similar effect (Wood and Ross, *Journ. Amer. Chem. Soc.*, 1942, **64**, 2759). It has also been observed that the hormone causes a fall in the phosphatase content of bone (Williams and Watson, *Endocrinology*, 1941, **29**, 250). These observations, together with the similarity of parathyroid hormone and insulin, suggest that the former may act enzymatically and that its action is related in some way to that of phosphatase. It has been suggested that vitamin D stimulates the action of the parathyroid glands, but the suggestion lacks confirmation. It is certain, however, that the ultimate effect of parathyroid hormone is to raise serum calcium level, probably by mobilising the calcium of bones. Even this may not be a direct action, and is possibly the result of an action on bone phosphate.

The essential fact is that the administration of parathyroid hormone results in a rise in serum calcium. The effect is gradual, and the calcium level rises to a maximum twelve to fifteen hours after administration. The mobilised calcium is derived from the bones, and long-continued administration is therefore inadvisable, as a dangerous degree of decalcification might be produced.

The parathyroids are small glands, and the usual number in humans appears to be four. Two occur in close proximity to the thyroid gland, and another two may be near, or even embedded in the thymus. The number of glands is variable, however, from individual to individual. If a subnormal

number is present, there is a considerable risk of them all being removed at thyroidectomy. This results in tetany from hypocalcæmia. For the relief of this condition, injection of parathyroid extract may be necessary indefinitely. Attempts have been made to find some other substance which could be given orally in place of this long-continued injection of parathyroid extract. The greatest success appears to have resulted from the use of dihydrotachysterol, but it has been questioned whether this substance is of any greater value than the related sterol, calciferol (vitamin D_2).

There is a superficial resemblance between the action of vitamin D and that of parathyroid hormone. Both substances raise blood calcium. In large doses vitamin D may do this by mobilising bone calcium as parathyroid does, but in normal doses it does so by promoting absorption of calcium from the intestine. The effect of the hormone on phosphorus metabolism is opposite to the effect of the vitamin. The hormone increases urinary excretion of phosphorus, whereas the vitamin decreases it.

Two types of cells have been noted in the parathyroid glands, " principal " and " oxyphile " cells, and it has been suggested that two hormones are secreted, one by each type of cell. The " principal " cells presumably secrete the calcium-phosphorus controlling hormone, but the function of the hormone from the " oxyphile " cells, if it exists, is quite unknown (*Journ. Amer. Med. Assoc.*, July 13, 1935, p. 113).

The known functions of the parathyroid are :—

1. To raise blood calcium and lower blood phosphorus
2. Possibly to increase ionised calcium in the blood
3. To increase urinary excretion of calcium and phosphorus
4. To obtain the calcium for this increased demand either from a large amount of ingested calcium or from the stores in bone

Hyperfunction of the parathyroid causes an excessive elevation of blood calcium and :—

1. The kidneys are damaged and, possibly as a result,
2. Blood phosphorus rises

3. Nitrogenous waste products accumulate in body fluids

4. Abnormal deposits of calcium salts occur in soft tissues

5. Kidney stones are formed because of high urinary calcium and phosphorus

6. Osteitis fibrosa cystica develops with an increase in activity osteoclasts and usually of osteoblasts. This results in generalised osteoporosis, cysts and giant cell tumours (*Journ. Amer. Med. Assoc.*, July 20, 1935, p. 197).

Clinical Uses of Parathyroid Hormone

Parathyroid extract is used chiefly for the relief of hypocalcæmic tetany and other hypoparathyroid conditions attributable to hypofunction of the gland or following parathyroidectomy. The extracts are rapid in action, and the effects are more lasting than those of calcium salts given intravenously. Particularly gratifying results have been obtained in the treatment of spasmophilia (infantile tetany). Such treatment, however, must not be of long duration, because it puts a severe strain on the already depleted calcium reserves of the bones.

Parathyroid therapy has also been used for the control of hæmorrhage (by increasing blood calcium) in hæmoptysis, purpura hæmorrhagica, and hæmorrhage following operation in jaundiced patients (vitamin K is specific in this instance). Somewhat inconclusive results have been reported following the use of parathyroid for the control of some of the symptoms of tuberculosis (*Journ. Amer. Med. Assoc.*, 1926, **86**, 1683, and *Amer. Rev. Tuberc.*, 1929, **20**, 901). A diuretic effect and some relief of symptoms have been reported in a few cases of eclampsia (*Surg. Gynec. and Obstet.*, 1929, **49**, 689).

These early findings have not been satisfactorily confirmed by subsequent clinical experience. Parathyroid hormone appears to be less certain in its action in controlling tetany than is vitamin D or dihydrotachysterol. Some authorities regard its clinical value with reserve, and others consider it to be useless. Zondek suggests that perhaps insufficient consideration has been given to dosage (" The Diseases of the Endocrine Glands ", Arnold, 1944).

Collateral administration of calcium by the oral route

appears to enhance the effect of parathyroid, and calcium should be given in most cases in order to inhibit excessive depletion of calcium from the body reserves.

Dosage and Administration of Parathyroid Hormone

Dosage of parathyroid hormone varies from about 10 units to 100 units daily. Whenever possible, it is advisable to control parathyroid dosage by frequent blood calcium estimations. Normal blood calcium is about 10 mg. per 100 c.c., and it is generally undesirable to give the hormone in doses which cause it to rise above 12 mg. A rise to 15 mg. may be dangerous.

After subcutaneous injection of parathyroid, the blood calcium begins to rise in about fifteen minutes, and a high level is maintained for about twelve to eighteen hours, after which there is a slow fall. Excessively frequent doses may cause vomiting and diarrhœa.

Average doses of parathyroid hormone are as follows :—

‡Post-operative tetany . . .	40 to 60 units daily
Infantile tetany (spasmophilia) . .	10 to 20 units daily
*Chronic ulcers	10 units daily
*Tuberculosis (secondary symptoms) .	10 to 20 units daily
*Hæmorrhage	10 to 20 units daily
*Eclampsia	10 to 40 units daily

‡ Only the more important suggested uses for parathyroid are mentioned, and some of these (marked *) are somewhat doubtful indications. Excellent results have been claimed, however, in eclampsia.

Parathyroid is best given in divided doses, usually at six- to eight-hour intervals. Intramuscular injection is generally most suitable, but in an emergency, when a rapid effect is required, the intravenous route is indicated. The subcutaneous route may be used instead of the intramuscular if desired.

Greene (*Practitioner*, March 1945, p. 157) gives the average dosage of parathyroid extract as 75 units twice daily in the treatment of tetany. The effect comes on in a few hours, and lasts for a day or two.

Hyperparathyroidism and the Effects of Overdosage

A description of some of the results of hyperfunction of the parathyroid glands has already been given in the section on the physiology of the glands.

Clinically, hyperparathyroidism or overdosage of parathyroid extract will be manifested in the first instance and in severe cases by anorexia, dullness, drowiness verging on coma, atonia and circulatory incompetence or failure. All these symptoms are a result of hypercalcæmia.

Administration of parathyroid extract over too long a period causes the development of a state of unresponsiveness. Thus administration must be limited to the early treatment of tetany, the treatment being continued with vitamin D and calcium as soon as possible. Alternatively, dihydrotachysterol may be given if this is obtainable. In treating tetany with parathyroid extract or dihydrotachysterol it is essential to give adequate amounts of calcium collaterally, in order to inhibit dangerous depletion of the calcium reserves of the bones.

CHAPTER V

INSULIN

THE story of the discovery and subsequent isolation of insulin is one of the most dramatic, and perhaps most widely known, in the whole history of endocrinology. The story began with the establishment of the relationship of the pancreas to diabetes by von Mering and Minkowski from experiments completed in 1889. From that time onwards, many attempts were made to prepare extracts of pancreas which were active in diabetes mellitus, but little success was obtained until the classic experiments by and under the direction of Banting were begun in 1921.

Banting used alcohol in preparing extracts, and this had the effect of eliminating trypsin. Trypsin had given rise to considerable difficulties for Banting's predecessors in their attempts to isolate insulin, and was probably the cause of the hormone not being isolated considerably earlier. Later, Banting found that acidified alcohol was of even greater value than plain alcohol. The extracts so prepared still contained appreciable amounts of protein, however, and were, in consequence, liable to cause sterile abscesses at the site of injection. The use of a stronger alcohol, as carried out by Collip, eliminated this. The strength of alcohol, finally used was 95 per cent. Insulin is not soluble in alcohol of this strength, but the water contained in the fresh minced pancreas reduces it to 50 to 60 per cent.—sufficiently dilute to dissolve insulin and sufficiently strong to precipitate the unwanted proteins.

By 1923 Banting and Best were able to report on the treatment of fifty patients with insulin, but still further improvements in its preparation were to follow. Dudley in 1923 proposed a method of isolation which gave a powder containing rather less than 50 per cent. of insulin, from which a relatively pure insulin can be prepared by precipitation as the picrate and final isolation as the hydrochloride.

Still other methods of extraction and concentration of insulin have been evolved, by Shaffer *et al.*, Scott, Blatherwick *et al.*, and a modification of Scott's method was described by Gerlough and Bates, and it is possible that further refinements still remain to be made in the process of isolating the pure substance on a commercial scale.

Chemistry of Insulin

Insulin is a protein or higher polypeptide with a molecular weight of about 35,000 to 37,000. The molecule may be spherical, and appears to be built up of the following :—

	Number of mols. per mol. of insulin
Lysine	6
Arginine	6
Histidine	18
Tyrosine	24
Cysteine	36
Glutamic acid	72
Leucine	84
Phenylalanine	1
Proline	10
Zinc	3
Amide amino	35
Sulphur	35

Recent work indicates that serine and threonine are also contained in the insulin molecule. There is some controversy as to the precise zinc content, but it seems to be generally agreed that zinc is an essential constituent. Possibly zinc is an active prosthetic group and insulin an enzyme. Figures varying between 0·3 and 0·6 per cent. have been found for the zinc content, and all can be reduced to 0·3 per cent. by repeated extraction with water.

An empirical formula of $C_{45}H_{69}O_{14}N_{11}S$ has been suggested for insulin, but the molecular weight suggests that the molecule must contain about thirty-five times these quantities of atoms.

Ninety-five per cent. of the sulphur content of insulin is accounted for by disulphide cystine.

Insulin can be reversibly or irreversibly inactivated. Reversible inactivation is produced by acetylation with acetic anhydride (activity restored by hydrolysis with dilute alkali). Irreversible inactivation is produced by reduction of –S–S–

groups with cysteine and glutathione, as well as by hydrolysis of peptide bonds with acids or alkalis, accompanied by decrease in cysteine or amino-nitrogen or both. Acetylation of amino-groups of insulin with ketene does not produce inactivation; prolonged interaction of insulin and ketene produces acetylation of phenolic OH groups. Reversible inactivation is produced by reactions involving a blocking of the amino or phenolic groups. It is concluded, therefore, that the hypoglycæmic activity of insulin is associated with certain dithio, phenolic and probably amino-groups, which are, however, found in most proteins. Thus Dixon (Thorpe's "Dictionary of Chemistry") concludes that "the activity is either a property of the molecule as a whole or is due to some labile unit which has hitherto escaped detection". This suggestion appears to leave out of account the possible significance of the zinc in the molecule. The protein may perhaps be looked upon as the pheron (bearer) fraction and the zinc as a prosthetic group of an enzyme analogous to the coenzyme (dehydrogenase ?) of the hexose phosphorylase in the carbohydrate cycle (see p. 209) containing magnesium as a prosthetic group.

Insulin is used clinically and is official as its hydrochloride. The pure crystalline substance is isolated as a routine on the commercial scale and issued for clinical use in the form of biologically standardised solutions of the hydrochloride in water.

Solutions of insulin (injection of insulin, B.P., 7th Addendum) for clinical use contain 20, 40 or 80 units per ml., and the solutions are adjusted to an acidity indicated by pH 3 to 4. Such solutions should be stored at a low temperature, above their freezing point, but below 20° C. (68° F.). Under these conditions, solutions of insulin may be expected to retain their potency for at least two years.

Protamine zinc insulin is the pharmacopœial synonym (7th Addendum) for injection of protamine zinc insulin. The solution is practically neutral (pH 6·9 to 7·3), and should be stored at a temperature not exceeding 20° C. as unmodified insulin. It may be assumed that globin zinc insulin should be stored at a similar temperature. If these conditions are observed, the modified insulins will also retain their potency for at least two years.

Insulin hydrochloride acts rapidly, and if large doses are given in an attempt to reduce the number of injections, a dangerous degree of hypoglycæmia is produced. Thus, from time to time attempts have been made to show up the absorption of insulin by various means. Suspensions of the hormone in oil were tried, but did not produce entirely satisfactory

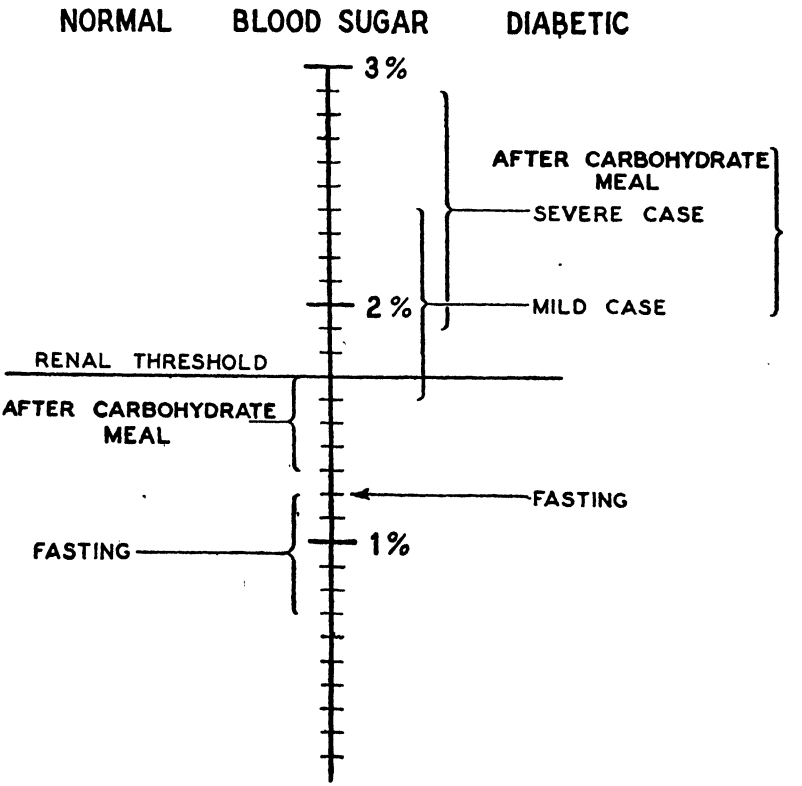


FIG. 1.—BLOOD-SUGAR LEVELS IN DIABETIC PATIENTS COMPARED WITH NORMAL LEVELS.

results. Aqueous solutions were issued which were precipitated by mixing with a solution of protamine in the presence of sodium phosphate buffer (" delay insulin " or " insulin retard ") before administration. Later it was found that a more satisfactory and more stable product could be made by adding a small amount of zinc sulphate. The suspension this formed

was more stable than " delay insulin " and could be issued ready precipitated. This preparation is now in common use under the name protamine insulin (with zinc) suspension, or P.Z.I. (protamine zinc insulin). Protamine zinc insulin is not an ideal preparation; for many patients its action is too delayed in onset and too prolonged. Further, an appreciable proportion of patients either have, or readily acquire, a hypersensitivity to protamine. Histone zinc insulin has been tried (*Brit. Med. Journ.*, March 14, 1942, p. 365), but has not come into general use. More recently, globin zinc insulin has been introduced, and is being used on a considerable scale. Unlike protamine zinc insulin, globin zinc insulin forms a clear solution. Its effect becomes apparent within two hours of administration, and reaches a maximum in two to eight hours. The effect can be detected up to about eighteen hours after injection, whereas the effect of protamine zinc insulin may go on for twenty-four to forty-eight hours. Thus the effect of globin zinc insulin is intermediate between that of unmodified and of protamine zinc insulin. Unit for unit, globin zinc insulin appears to be slightly more active than the latter.

Mode of Action of Insulin

Some indication of the limited knowledge of the chemistry of the action of insulin has been given in the section on the chemistry of the hormone itself.

The rôle of insulin in body function is best understood from a consideration of the carbohydrate exchanges which precede the oxidative breakdown of glucose in the muscles (see page 209). These preliminary exchanges are represented diagrammatically in Fig. 2 opposite.

The main store of carbohydrate is in the form of liver glycogen. This is formed from dietary carbohydrates and from proteins and fats. The transport form of carbohydrate is glucose, and in this form it is conveyed to muscles to form secondary stores of muscle glycogen, and some goes directly to peripheral tissues for immediate utilisation. The oxidation of glucose in muscle tissues results in the formation of lactic acid, a considerable proportion of which is utilised in the liver to form more glycogen.

In a normal person these exchanges are so co-ordinated

that the blood glucose is kept at about 0·1 per cent. (0·07 to 0·12 per cent.) when fasting, and rising to 0·13 or 0·17 per cent. for an hour or two after a meal. This control of blood-sugar level is largely dependent on insulin and on adrenaline, and to a

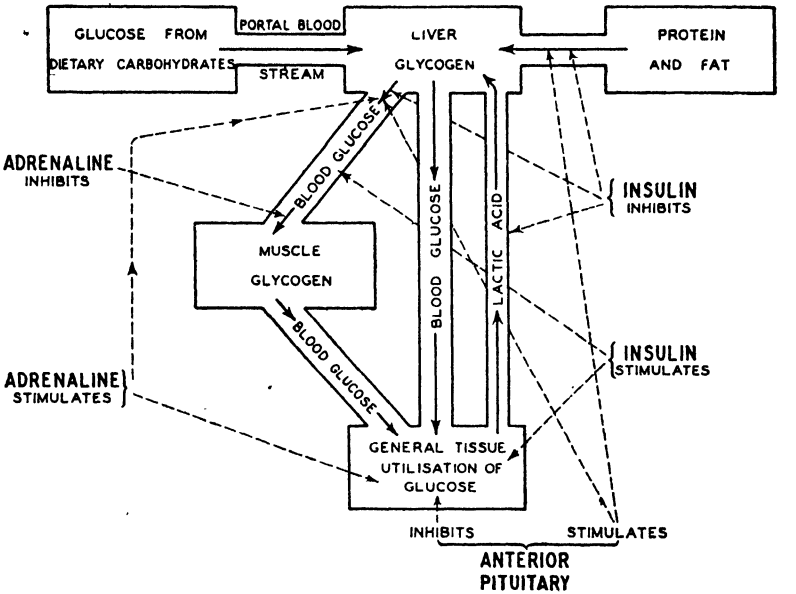


FIG. 2.—THE CARBOHYDRATE EXCHANGES WHICH PRECEDE UTILISATION OF GLUCOSE IN MUSCLE TISSUE.

less extent on anterior pituitary secretion. Insulin tends to decrease blood glucose ;

(1) by inhibiting glycogen formation from
 (*a*) protein and fat,
 (*b*) muscle lactic acid ;

(2) by *inhibiting* formation of blood glucose from liver glycogen ;

(3) by *stimulating* formation of muscle glycogen from blood glucose (principal action) ; and

(4) by *stimulating* utilisation of glucose by tissues generally.

Adrenaline is largely, but not entirely antagonistic to insulin. Like insulin, adrenaline stimulates utilisation of glucose by tissues generally. On the other hand, adrenaline

stimulates the formation of glucose from liver glycogen and inhibits the conversion of this glucose into muscle glycogen. Thus insulin has a hypoglycæmic action, whereas adrenaline produces a hyperglycæmic state. The anterior pituitary introduces a further complication, in that it inhibits tissue oxidation of glucose and stimulates the formation of liver glycogen from muscle lactic acid and from protein and fat (hypoglycæmic action). So far as is known, the secretion of insulin, unlike that of most hormones, is not influenced by any of the factors of the anterior pituitary.

Potency and Units of Insulin

The original unit of insulin was the amount which is required to reduce the blood-sugar of a 4 lb. rabbit to 0·045 per cent. The present clinical or international unit is one-third of this amount. A milligram of insulin has an activity of 22 international units.

The hormone is standardised by comparison with a known standard. The standard and the unknown are administered to two groups of rabbits, and the average percentage falls of blood-sugar are noted. A few days later the group of animals which received the standard are given the unknown and vice versa, and the results are compared.

Administration of Insulin

The chemical nature of insulin, it will be seen from the foregoing, is such that oral administration must of necessity be ineffective. Being a polypeptide, if not a protein, insulin is inactivated by the gastric juices before it can be absorbed. Indeed, the size of its molecule is such that it could perhaps not be absorbed at all from the alimentary canal without being first broken down into smaller constituents, and thus inactivated. Thus if, it is to be effective, insulin must be administered by injection. This applies also to the various modified insulins which have been introduced from time to time, some of which have come to be used almost as extensively as unmodified insulin.

Insulin, modified or unmodified, is injected subcutaneously. Intradermal or intramuscular injections are painful.

Clinical Uses of Insulin

Insulin is now used almost exclusively for the treatment of diabetes mellitus. The treatment is not curative, but substitutive. It must therefore be continued for the remainder of the patient's life. It is possible that remissions have occurred during treatment in some mild cases, but such remissions are rare and not to be expected.

The object of administration is to enable the patient to maintain a normal blood-sugar level, and so lead a more or less normal life. This can be achieved only if a calculated dietary regimen is adopted and balanced with a carefully determined dosage scheme of insulin, modified or unmodified as indicated. The diet and the insulin dosage must be determined individually for every patient on the lines indicated in the literature issued by the manufactuers of insulin and in the books devoted to the subject. The detailed information is available in R. D. Lawrence's " The Diabetic Life " (Churchill, 1941), to which a war-time supplement has been issued.

The first symptom of diabetes, glycosuria, may pass unnoticed for some time, but as the disease progresses the patient may complain of lassitude, polyuria, thirst after meals, increasing hunger and loss of weight. The thirst after meals is symptomatic of hyperglycæmia, which usually reaches 0·25 per cent. before these symptoms appear. In spite of a considerable fluid intake the polyuria results in dehydration, indicated by a drawn appearance, cramps at night, dry skin subject to eczema and boils. Constipation is usual, general body weakness increases and temperature becomes subnormal. The eyes and nervous system may be affected, and in old people gangrene, particularly of the legs, may appear. If nausea, anorexia, vomiting and abdominal pain appear, ketosis must be suspected. This results from disordered fat metabolism, and may be shown by a characteristic odour of the breath. This is described as resembling violets or new-mown hay, and is due to the presence of acetone. Acetone and aceto-acetic (diacetic) acid can be detected in the urine.

A patient who has all or a number of these symptoms is in need of immediate treatment. If treatment is withheld, drowsiness or breathlessness develops and the patient is in danger of going into a deep coma and dying.

Immediate treatment consists in the administration of ummodified insulin. The patient becomes normal with dramatic suddenness.

Thereafter the patient must be thoroughly investigated and stabilised on a suitable diet and dose of insulin.

In most cases the patient is taught to administer the insulin for himself and to carry out a test of the urine for sugar daily or when necessary. The modified insulins (protamine zinc insulin and globin zinc insulin) are not indicated for the treatment of marked degrees of hyperglycæmia, but only for the maintenance of patients who have been stabilised on an appropriate diet.

Dosage of Insulin

As has already been indicated, the dosage of insulin must be determined for each individual patient. Accordingly, no pharmacopœial dose is given. Generally, 5—30 units are required daily, but in severe cases, and for the treatment of diabetic (hyperglycæmic) coma, larger doses may be necessary.

Insulin in Non-Diabetic Conditions

Insulin treatment has been suggested for debility, emaciation and malnutrition. Dosage varies, but patients under close observation may be given 5 units three times a day, increasing by 5 units a day until 20 or 30 units are being given three times daily. Ambulatory patients should not receive more than 20 units, or in favourable circumstances, 40 units, daily, and in all cases each dose must be followed by an adequate meal or by the ingestion of glucose. No injection should be given after 7 p.m., in order to avoid the risk of a hypoglycæmic reaction. Treatment should be continued for four or five weeks.

Insulin has also been given to produce hypoglycæmic shock in the treatment of schizophrenia. This method has been generally abandoned, however, except in modified forms. Hill still recommends the treatment using a mixed injection of insulin with histamine in increasing doses. More frequently, leptazol is used to produce the convulsions, and even this is being superseded by treatment with electrically induced convulsions.

CHAPTER VI

PITUITARY HORMONES (POSTERIOR LOBE)

THE function of the pituitary was originally thought to be the secretion of nasal mucus. Thus it received its name from *pituita*, meaning mucus or phlegm.

Although the pituitary gland as a whole probably secretes more distinct hormones than any other gland, the posterior lobe probably secretes only two or three hormones. The histological structure of the gland, in which the two lobes are readily discernible, suggested, early in the investigations of its endocrine properties, that the two lobes probably had distinct functions. The importance of the gland as a whole was not generally accepted, however, until comparatively recently. As late as 1922, Walton stated that in two patients complete extirpation of the gland did not produce any marked untoward effects. He concluded from this that the pituitary gland had no functions which could not be taken over by other glands (*Brit. Med. Journ.*, 1922, II, 835). Two separate functions of the posterior lobe of the pituitary gland had been recognised for many years: an effect on blood pressure and an effect on uterine muscle. Martindale (" Extra Pharmacopœia ", 1920), among others, suggested that these effects were possibly due to two distinct active principles. It was not until 1927, however, that Kamm, Aldrich, Grote, Rowe and Bugbee announced the separation of the two hormones, an oxytocic and a pressor principle.

Bourne and Burn (*Lancet*, Oct. 6, 1928) confirmed the oxytocic activity of the one and the absence of oxytocic effect in the other, although Geiling (*Journ. Amer. Med. Assoc.*, March 2nd, 1935, p. 738) did not agree that separation of the two factors had been achieved even then. Whole posterior pituitary extract and the pressor fraction, vasopressin, both exert an anti-diuretic effect, but Bijlsma, Burn and Gaddum (*Quart. Journ. Pharmacy*, 1928, Vol. I, 494) suggest that there is a separate antidiuretic hormone which has not yet been separated from the pressor principle.

Chemistry of the Posterior Pituitary Hormones

The posterior lobe of the pituitary gland is formed from a downward growth from the base of the brain. As is to be expected, therefore, its structure bears a distinct resemblance to nerve tissue.

It seems likely that the two hormones secreted by the posterior lobe are relatively simple, probably polypeptide, basic substances.

The oxytocic hormone has been named α-hypophamine or oxytocin, and is available for clinical use in a comparatively pure form under the proprietary name " Pitocin ". The other hormone, β-hypophamine, or vasopressin, is also available in highly purified form under the name " Pitressin ".

Neither of these preparations is official, the only pharmacopœial preparation being an acid aqueous extract of the whole posterior lobe which has both the oxytocic and the vasopressor activity.

α-Hypophamine is appreciably soluble in organic solvents, particularly ether and acetic acid. β-Hypophamine is freed from inert protein with acetone and is precipitated with ether. A more detailed account of this separation is given in Harrow and Sherwin's " Chemistry of the Hormones " (Baillière, Tindall and Cox, 1934). α-Hypophamine is a white, stable substance, soluble in water and eighty times as potent as the international standard pituitary powder. β-Hypophamine is also a white powder with similar properties, but one hundred and fifty times as potent as international standard pituitary powder,

Unit and Potency of Posterior Pituitary Hormones

The international standard pituitary powder consists of fresh posterior lobe substance of oxen, defatted with acetone and dried. Half a milligram of this powder has an activity of one international unit.

The potency of pituitary (posterior lobe) extracts is estimated by comparison of an acid alcohol extract prepared by a prescribed method (vide B.P. 1932, Appendices) so as to contain two international units per ml. The test is carried out on isolated strips of virgin guinea-pig uterine muscle.

Certain German preparations have been standardised in

terms of Voegtlin units; these are equal to international units.

Physiology and Mode of Action of Posterior Pituitary Hormones

Nothing is known of the chemistry of the action of posterior pituitary hormones, although it is possible that, in common with the other polypeptide and protein hormones, their action is enzymatic in nature.

Although the two principal hormones, α- and β-hypophamine, have been separated and are available for general clinical use, an extract of the whole posterior lobe containing both these hormones is in much more common use.

The most marked action of the official extract is its stimulation of smooth muscle, especially that of the uterus. By reason of this action, pituitary (posterior lobe) extract was originally used in order to accelerate labour. The action is so vigorous, however, that there is some risk, either of injuring the infant or of rupturing the uterus. In consequence, this use of the extract was practically abandoned. More recently (1944, 1945) there has been some tendency to reintroduce this use of pituitary in a modified form for certain cases. Administration must be cautious, and small doses are given at relatively frequent intervals.

A second, and probably the most important use for pituitary extract is the control of post-partum hæmorrhage. The extract is administered after the delivery of the placenta. This produces an almost immediate contraction of the uterus, which continues for some time, and so provides an effective means of limiting the hæmorrhage.

The action on smooth muscle is utilised also to induce peristalsis in cases of paralytic ileus.

Posterior pituitary also has a vasoconstrictor action the effect of which is to raise blood pressure. This property may be employed to combat minor degrees of shock.

The muscle of the bladder is stimulated and the bronchial muscle is relaxed by the extract, so that bronchial asthma may be relieved by it.

It retards the peripheral utilisation of blood-sugar, and so may be used for the relief of hypoglycæmic coma.

Posterior pituitary is also the principal medicament em-

ployed to control the diuresis of diabetes insipidus. There is reason to believe that the antidiuretic action is attributable to a specific hormone, distinct from α- and β-hypophamine. The site of action of this hormone is not clear. It may act upon the regulatory centres in the diencephalon or directly upon the kidneys (renal epithelia), or perhaps both diencephalon and kidneys are affected.

Undoubtedly, pituitary (posterior lobe) extracts act upon regulatory centres in the central nervous system, and much more marked effects follow its administration intrathecally than subcutaneously.

Just which of these actions are attributable to α-hypophamine and which are produced by β-hypophamine is not yet entirely clear. α-Hypophamine is the oxytocic principle, and it may also be the factor which corrects hypoglycæmia. The vasopressor activity is undoubtedly exerted by β-hypophamine. The antidiuretic factor, it has been suggested, may be secreted by the pars intermedia (hence it has been called " intermedin "). It should be noted, however, that the pars intermedia is hardly developed in man. In the lower vertebrates it is more highly developed, and appears to be the site of secretion of the hormone controlling the melanophores in frog's skin. This also has been called intermedin, but it is not known whether the two factors are identical.

These inconclusive observations appear to be clarified to some extent by the observations of Mukherjee (*J. Obst. and Gynæc. Brit. Emp.*, Oct. 1941, p. 586). Mukherjee reports a significant degree of correspondence between the melanophoric, antidiuretic and pressor effect on frogs of ultrafiltrates from the blood of patients with pregnancy toxæmia. His findings seem to indicate that all three of these effects are exerted by vasopressor substance, and that pregnancy toxæmia is the result of hypersecretion of this hormone together with a hypersensitivity of the patient towards it.

Clinical Uses, Dosage and Methods of Administration

The principal clinical uses of posterior pituitary preparations have already been mentioned : control of post-partum hæmorrhage, of diuresis of diabetes insipidus, relief of paralytic ileus and of asthmatic paroxysms. It may also be employed in

missed abortion and uterine inertia. It has been used to raise blood pressure in shock and for the correction of hypoglycæmia following over-doses of insulin.

Vasopressin and oxytocin have opposite effects on intestinal peristalsis, the former stimulating it and the latter inhibiting it. Thus vasopressin is said to be preferable to preparations of the whole posterior lobe in intestinal atony.

Post-operative urinary retention has also been relieved with pituitary (posterior lobe) extract, but the synthetic parasympathomimetic, carbachol, is now generally employed for this purpose.

The effect of posterior pituitary appears to be more prolonged than that of adrenaline in relieving the spasms of asthma. The former may be used alone, or both may be administered collaterally to produce an enhanced effect.

Diabetes insipidus appears to be the result of posterior pituitary insufficiency, and both the polyuria and the intense thirst of this disease can be controlled by extracts of the whole lobe. Continuous application produces the best results, so that intra-nasal application is generally preferable to injection. Three methods of intra-nasal application have been employed : insertion of pledgets of cotton wool soaked in posterior pituitary extract, the use of snuff containing dry gland substance, and the use of nasal jelly containing the extract. Either vasopressin or whole posterior lobe preparations are effective in diabetes insipidus, but the extract of the whole posterior lobe must be employed for the induction of labour (see dosage table).

For this last purpose, pituitary extract (20 minims) is soaked up in a pledget of cotton wool and inserted into one nostril between the septum and the inferior turbinate. After one or two hours this pledget is removed, and a similar one is inserted in the other nostril.

In diabetes insipidus the use of pituitary snuff is effective, and at the same time convenient for the patient. Mainzer (*Brit. Med. Journ. Epit.*, [i], 1935, p. 72) suggested a snuff containing posterior lobe substance equivalent to 1000 units per gramme. An average dose is 15–90 units, and this is given three times a day. The diluent may be some inert powder, such as kaolin, magnesium carbonate, or lycopodium. Metz and

DOSAGE OF POSTERIOR LOBE PITUITARY PREPARATIONS

Condition	Dosage of		
	Pituitary Extract	Vasopressin	Oxytocin
Hæmorrhage (post-partum)	0·5 c.c. by slow intravenous injection or 0·5 c.c. intramuscularly followed by 0·5 c.c. after delivery of placenta	—	For patients with nephritis, myocarditis, eclampsia or arteriosclerosis. Dosage as for pituitary extract
Hæmorrhage (surgical, prevention of)	0·5 to 1 c.c. ½ hour before operation	For patients with hypotension or who may become surgically shocked	For patients who may be subject to pituitary shock (*i.e.* dehydrated patients)
Herpes zoster	1 c.c. intramuscularly	—	—
Labour (induction of)	0·2 to 0·5 c.c. subcutaneously after castor oil (1 oz.) and quinine (10 grains), orally and œstrogenic hormone (*q.v.*), *or* pituitary intranasally (see p. 53)		
Labour (second stage)	0·1 to 0·2 c.c. intramuscularly, repeated as necessary.	—	0·1 to 0·2 c.c. in eclamptic or dehydrated patients
Labour (third stage)	0·5 c.c. intramuscularly.	—	0·5 c.c. for eclamptic or dehydrated patients
Cæsarean section	1 c.c. into posterior wall of uterus immediately after delivery of infant	—	—
Paralytic ileus (or intestinal atony)	0·2 to 0·5 c.c. intramuscularly		
Shock	0·15 to 1 c.c. well diluted with normal saline solution intravenously in severe cases. 0·5 to 1 c.c. intramuscularly in less severe cases	—	—

Lackey (*Amer. J. Digest. Dis.*, 1940, 27) have suggested undiluted posterior pituitary substance by intra-nasal insufflation for peptic ulcer. The dose was ¾ grain four times daily half an hour after food and at bed-time.

Intra-nasal administration of pituitary preparations has become a more or less standard method of treating diabetes insipidus, but attempts have also been made to control the speed of utilisation by various means, analogous to those which have been applied successfully to insulin. A concise but informative review of many such attempts was given in the *Lancet* of February 27, 1943, p. 265. Among the preparations used were concentrated pituitary extract in oil with wax and wool-fat, vasopressin with 0·1 per cent. of zinc acetate, pellets of dried posterior pituitary substance for subcutaneous implantation, vasopressin suspended in pea-nut oil and vasopressin precipitated with tannic acid and suspended in pea-nut oil. A dose of 0·2 c.c. is given daily by intramuscular injection, and subsequent doses are increased or decreased in size or frequency according to the response of the patient. Each c.c. of suspension represents five pressor units. This method of treatment was reported on earlier by Green and January (*Journ. Amer. Med. Assoc.*, Oct. 5th, 1940, p. 1183). Dieckman and Kharasch (*Amer. Journ. Obst. and Gynecol.*, Nov. 1942) report on the use of posterior pituitary "sulphonate" in obstetrics as a means of prolonging and mitigating the violent effects of the oxytocic hormone.

It appears to be generally agreed that the utmost caution must be observed when posterior pituitary preparations are given for the induction of labour or during any stage of labour before the birth of the placenta.

CHAPTER VII

PITUITARY HORMONES (ANTERIOR LOBE; ALSO CHORIONIC AND SERUM GONADOTROPINS)

THE early history of the pituitary gland has been briefly indicated in the previous chapter. The two distinct lobes of the pituitary body, posterior and anterior, gave an indication early in the investigation of the gland that the two lobes probably had quite distinct functions. The origin of the pressor and oxytocic properties was found to be in the posterior lobe, and a factor controlling growth was found to originate from the anterior lobe. Hypersecretion of the acidophil cells appears to be the cause of gigantism, in which there is an overall growth of the body to an abnormal size, but in which normal proportions are retained. This is the effect produced by the growth hormone, which has been called somatotrophin. Adenoma of the acidophil cells causes acromegaly, a condition characterised by overgrowth of extremities and prominent parts of the body, and usually not appearing before the third decade of life. Following the postulation of the growth hormone and the proof of its existence by Evans and Long in 1921, a number of factors with diverse functions were postulated. A gonadotropic hormone was demonstrated by Smith and Engel in 1927, and a hormone controlling fat metabolism by Anselmino and Hoffmann in 1931. Numerous factors controlling carbohydrate metabolism have been reported. Undoubtedly there are several such factors, but the existence of all those postulated has not been confirmed. The existence of the thyrotropic hormone was established in 1930, and in the same year Corner pointed out that secretion of milk is brought about by one of the pituitary hormones. This was prepared by Riddle, Bates and Dykshorn (1932, 1933), and named prolactin by them. In addition, a mammotrophin, causing growth of the mammary glands, has been postulated.

A parathyrotropic action of anterior pituitary extract was

demonstrated in 1934 by Hertz and Kraues and by Anselmino and Hoffmann.

Evans *et al.* in 1932 showed that anterior pituitary extracts were capable of preventing the adrenal atrophy produced in hypophysectomised animals, and in the following year Collip, Anderson and Thomson stated that the effect was not produced by gonadotropin, thyrotropin or prolactin. The hormone has been called variously, adrenotrophic, interrenotrophic and corticotrophic hormone.

The gonadotropin (? gonadotropins) of the anterior lobe of the pituitary gland has probably been the subject of more intensive investigation than any other hormone secreted by this gland, but knowledge of it is still far from complete. Further, the relationship between the pituitary hormone and the gonadotropins of pregnant mare's serum and human pregnancy urine is still obscure. A certain amount of progress has been made in experimental research, but clinical results have been disappointing.

Smith, Zondek and Aschheim discovered independently in 1926 that implantation of anterior pituitary tissue into infantile mice produces precocious sexual maturation, including follicular ripening and ovulation. Enlargement of the pituitary gland during pregnancy led to the finding of ovary-stimulating substances in the body fluids and urine of pregnant women. This formed the basis of the Aschheim–Zondek pregnancy test. Zondek in 1929 found a gonadotropic substance in the urine of menopausal women which had a limited follicle-stimulating action. This substance is probably of pituitary origin, whereas the gonadotropin of pregnancy is almost certainly of chorionic origin and is more luteinising than follicle-stimulating. These two substances were named by Zondek " prolan A " and " prolan B ", respectively. It is now commonly accepted that pituitary gonadotropin consists of two substances corresponding approximately to prolans A and B. Prolan A and the pituitary follicle-stimulating hormone of menopausal urine may be identical or closely related. It has been assumed that serum gonadotropin (from pregnant mares) has the same action, and that it, too, may be identical with prolan A or the follicle-stimulating pituitary hormone. The results of the clinical use of serum gonadotropin have been somewhat disappointing, and

this may indicate that prolan A, F.S.H. and serum gonadotropin are not necessarily identical.

Prolan B (interstitial cell-stimulating hormone, I.C.S.H.) and chorionic (pregnancy urine) gonadotropin are similar, but there are both chemical and physiological differences between the two substances.

It will be seen that much remains to be learnt about the pituitary gland and its secretions. Young concludes an interesting and informative review of the subject (*Practitioner*, March 1945, p. 129) with the following: "Experimental and clinical research have already provided much knowledge concerning the pituitary gland. But still more is required before the tools which are now being forged can be utilised with maximal efficiency."

Chemistry of Anterior Pituitary Hormones

Little or nothing is known of the chemical structure of the pituitary hormones secreted by the anterior lobes. They are possibly proteins or polypeptides of varying degrees of complexity. The pituitary gonadotropin(s) are little understood, and its (their) relationship to serum and chorionic gonadotropins remains obscure. The few known chemical properties of the various hormones are summarised below.

1. *Growth Hormone* ("*Antuitrin G*", *Somatotrophin*). An unstable substance, extracted from the gland with alkali and precipitated with acetone after neutralisation. At this stage, gonadotropin has not been separated, but precipitation with diluted flavianic or trichloracetic acid removes this and most other hormones. Growth hormone is soluble without decomposition in strong acetic acid which destroys gonadotropin.

2. *The "Galactins."*

(a) *Prolactin* ("*Physolactin*"). This hormone is extracted by alkali from the gland and precipitated by acid. The process is much more complicated than appears from this statement; details were given in Harrow and Sherwin's "Chemistry of the Hormones", 1934.

(b) *Mammatrophin.* The existence of this hormone is doubtful, and chemical properties have not been suggested.

3. *Metabolic Factors.*

(a) *Carbohydrate Factors* ("*Glycotrophins*"):—
Diabetogenic (Houssay).
Glycosuric (Young).
Hyperglycæmic (Anselmino and Hoffman).
Pancreatotrophic (Anselmino and Hoffman).
Contra-insulin (Lucke).

(b) *Fat Metabolism Factors*:—
Ketogenic (Burn–Ling).
Lipoitrin (Raab).

The knowledge of the chemistry of these factors is so far negligible in amount, and even the existence of a number of them is controversial.

Houssay's diabetogenic hormone is soluble in water and in 60 per cent. alcohol, but insoluble in absolute alcohol and the usual organic solvents. It is readily adsorbed, and may thus be adsorbed on kaolin from urine, particularly the urine of diabetics, and eluted from the kaolin with dilute alcohol.

Collip (*Journ. Amer. Med. Assoc.*, March 16, 1935, p. 916) assumes that the diabetogenic action of the pituitary is the result of the combined action of two factors, one which acts on blood-sugar levels (Houssay's diabetogenic factor), and one which produces ketosis (ketogenic factor of Burn and Ling ?).

(c) *Thyrotropic Hormone* (*Thyrotrophic Hormone*).—This hormone can be extracted from the gland by weak acid or weak alkali (0·1 per cent. acetic acid or sodium hydroxide). The solution is adjusted to pH 7·8, and then filtered through a Seitz bacterial filter (Harrow and Sherwin, 1934). Krogh states that the hormone is soluble in water, Ringer's solution and in 48 per cent. alcohol, but insoluble in 70 per cent. alcohol.

4. *Gonadotropin.*—Pituitary gonadotropin is excreted much more slowly from the body than chorionic gonadotropin. This may possibly indicate that it possesses a larger molecule. Like insulin, pituitary gonadotropin is enhanced in its action by such substances as zinc sulphate and tannic acid. Pituitary gonadotropin is secreted by the basophil cells, and is extracted from the gland by acids and alkalis, and subsequently purified

by adsorption and dialysis. It is resistant to decomposition by dry heat, but in the presence of traces of moisture it is inactivated at 62° C. Strong acids and alkalis decompose it, and it is insoluble in organic solvents.

As has already been stated, pituitary gonadotropin consists of two factors. These have been separated and prepared in a state of comparative purity, but there is little information available on the chemical differences of the two substances, beyond the fact that the follicle-stimulating factor is more soluble in water than the luteinizing factor.

" Prolan B " has been prepared from placental substance with 20 per cent. aqueous sulphosalicylic acid solution and from pregnancy urine by precipitation with alcohol. The alcohol precipitate is purified with phosphotungstic or phosphomolybdic acid, and subsequently by electrodialysis.

Methods of Estimation of Anterior Pituitary Hormones and of Serum and Chorionic Gonadotropins

Growth Hormone.—The potency of an anterior pituitary preparation is estimated by comparison of the unknown with a preparation chosen as a standard using hypophysectomised rats. Details of carrying out the test, as well as the preparation of the rats by hypophysectomisation, are described by J. H. Burn (" Biological Standardisation ", Oxford, 1937). There is no international unit of activity.

Thyrotrophin.—There are two methods of standardisation of thyrotophin, guinea-pigs being the test animal in both instances. If the potency of a preparation is expressed in " guinea-pig units " it has probably been standardised in terms of its power of effecting certain histological changes in the thyroids of the test animals as described by Aron, Loeser, Sevringhaus and Heyl. This method is somewhat inaccurate. The most satisfactory method of standardisation is that of Rowlands and Parkes, wherein the potency is expressed in terms of " guinea-pig weight units ", and refers to the effect of the preparation in causing increase in weight in the thyroids of guinea-pigs. The method is described by Burn (*ibid.*), or Rowlands and Parkes' original paper may be consulted (*Biochem. Journ.*, 1934, 28, 1829).

Prolactin.—An international unit has been defined for this

hormone, and this should replace the Riddle unit previously employed. The international unit was intentionally formulated so as to be virtually identical with the Riddle unit. The test animal is the pigeon (male or female), and the reaction is the crop-gland stimulating activity of the hormone. An international unit is defined as the specific activity contained in 0·1 milligram of the standard preparation. Again Burn may be consulted for the details of the method of estimation, or the original paper by Riddle, Bates and Dykshorn may be consulted (*Amer. Journ. Physiol.*, 1933, **105**, 191).

Serum Gonadotropin (Equine).—Estimations of the potency of serum gonadotropin from pregnant mares may be carried out satisfactorily by various methods. The standard preparation and the hormone of unknown potency are administered to the test animal by the subcutaneous route. The test animal is usually the immature female rat, but mice, rabbits and male rats have been employed. The following effects have been considered by various investigators :—

1. Increase in ovarian weight
2. Occurrence of vaginal cornification alone *or*
3. Together with ovarian and uterine changes
4. Formation of corpora lutea
5. Changes in uterine weight together with the incidence of vaginal cornification in female rats or mice
6. Increase in weight of the seminal vesicles of male rats
7. Production of ovulation on intravenous injection to female rabbits

On the whole, it appears that the most accurate method is that involving the determination of increase in ovarian weight, but the other methods appear to be sufficiently accurate to permit of their being used if desired.

The international unit for serum gonadotropin is the specific gonadotropic activity of 0·25 mg. of the standard preparation.

Chorionic Gonadotropin.—A small but seemingly constant amount of follicle-stimulating substance appears to be present in the chorionic luteinising substance prepared from human pregnancy urine. Estimation of this follicle-stimulating substance is regarded as the most accurate method of estimating the potency of the luteinising fraction.

Immature female rats are used almost exclusively as the test animals. The changes observed are increase in weight of the ovaries and the production of vaginal cornification.

The international unit of chorionic gonadotropin is defined as the specific gonadotropic activity of 0·1 mg. of the standard preparation.

Anterior Pituitary Gonadotropins.—In order to assist in research, an international standard preparation of unfractionated anterior pituitary gland substance has been prepared but international units of potency have not been defined in terms of this preparation.

Mode of Action of Anterior Pituitary Hormones

In consequence of the limited knowledge of the chemical nature of the anterior pituitary hormones, little is known of the chemistry of their action in the body. It is possible that there is a close chemical similarity between the various hormones, and this may be, in part, the explanation of how such a small gland as the pituitary is able to produce such a bewildering variety of substances with such different actions. Zondek, ("Diseases of the Endocrine Glands", 1944) has suggested that "Probably these various substances are but *modifications, derived from a chemically uniform basic substance by comparatively insignificant molecular changes.*"

Growth Hormone.—Normally growth hormone promotes growth generally, but, for some reason as yet not understood, it sometimes produces peculiar local growth or localised acromegaly, as in lymphangiectatic gigantism, in which perhaps only the feet and one leg are involved. Hypersecretion of growth hormone beginning after normal growth has ceased produces true acromegaly, which is manifested by abnormal enlargement of the face and extremities. Growth hormone almost certainly exerts its action in normal and pathological states by virtue of its effect on nitrogen metabolism. It causes a decrease in nitrogen excretion. In rats, at least, it decreases the amino-acids, urea, ammonia and non-protein nitrogen in liver and muscles and in the total metabolism. Apparently it causes improved utilisation of nitrogenous substances and increases deposition of protein.

Thyrotrophin.—The suffix "trophin" is used for this factor

because there is some possibility of its being in a sense a "nutrient", rather than a stimulant, of the thyroid gland. Like the other factors, however, the chemical and other details of its mode of action remain unknown. The secretion of thyrotrophin is inhibited by the thyroid hormone, and when secretion of the latter is diminished the thyroid gland becomes enlarged under the influence of the thyrotrophin. This is probably of the nature of a compensatory hyperplasia, and represents an attempt to correct the thyroglobin deficiency by providing extra thyroglobin-secreting tissue. Thyroid hyperplasia (goitre) is produced when the antithyroid substances (sulphonamides, thiouracil etc.) are given to normal animals or to hyperthyroid animals in excessive doses. Di-iodotyrosine has been credited with antithyroid properties, and it might be expected to inhibit thyroid hyperplasia in spite of this, since it is a precursor of thyroxine and a constituent of thyroglobulin. It has been stated, however, that di-iodotyrosine, given collaterally with thiourea or thiouracil, does not inhibit thyroid hyperplasia (*Journ. Clin. Endocrinol.*, May 1944, p. 213). It seems desirable that this point should be further investigated.

Thyrotrophin is indicated for the treatment of hypothyroid states in which there is atrophy of the thyroid gland in addition to hyposecretion of thyroid hormone. Hypothyroidism with goitre is an indication for the administration of iodine or of thyroxine or thyroid gland substance. The goitre in such cases is an indication that there is no pituitary deficiency, and to give thyrotropin would result in further enlargement, whilst the hypothyroid state would not be relieved. Thus thyrotropin is indicated (in the absence of thyroid enlargement) in obesity and conditions of lowered metabolic rate, notably Simmonds' disease. In this last condition it is generally an advantage to give chorionic gonadotropin in small doses collaterally in order to correct any associated hypogonadism.

Prolactin.—The name "galactin" was originally suggested for this factor, but it is now almost invariably known as prolactin. In view of its action on the corpus luteum (see under "Gonadotropins"), it has also been called "luteotropin", but this name has not come into general use.

Prolactin is one of a number of factors concerned in the

function of lactation. Certain cases of deficient lactation are therefore likely to benefit from its administration, but it is not yet possible to differentiate between the cases which will and which will not respond. The clinical results of the use of prolactin have thus so far been somewhat inconclusive and disappointing. In the future, when it is possible to determine more precisely the causes of deficient lactation, prolactin will undoubtedly take its place among the specific remedies for the condition. There seems to be little doubt also that prolactin will be found to have an important rôle in conjunction with the gonadotropins, particularly in connection with the corpus luteum. As will be seen later, knowledge of the gonadotropic substances is far from complete, and it is probably not too much to suggest that carefully controlled collateral use of prolactin with the gonadotropins may do much to elucidate the precise actions of the latter.

Other functions of prolactin include some control over general body growth and the development of normal-sized body viscera. It appears to stimulate luteal secretion, but it also has an anti-gonadotropic effect. This last property has been utilised to control abnormal uterine bleeding attributable to excessive secretion of œstrogenic hormone. Prolactin is thought to inhibit secretion of the follicle-stimulating hormone of the anterior pituitary, and indirectly, therefore, the œstrogenic hormone. Inhibition is not complete, even when doses sufficient to produce amenorrhœa are given, for vaginal smears indicate a degree of œstrogen suppression comparable with those produced by androgens or X-rays, and the rhythm of the cycle is not disturbed.

The use of prolactin is stated to be completely harmless. Unlike X-ray treatment, it cannot produce permanent amenorrhœa or sterility, its action is considerably more quickly exerted than that of chorionic gonadotropin, and it can have no virilising effect, as the androgens have. Prolactin decreases the duration of prolonged menstrual periods and the volume of excessive flow. It increases the intermenstrual interval where this is abnormally short, and it prevents ovulatory hæmorrhage. Dysmenorrhœa associated with menorrhagia is relieved, premenstrual bruising is diminished. The premenstrual bruising is thought to be a manifestation, as is the menorrhagia, of an

excess of a bleeding factor produced by the spleen which seems to have a pituitary-inhibiting action.

This antimenorrhagic effect of prolactin is the subject of a paper by Goldzieher in the *Journ. Clin. Endocrinol.*, March 1945, p. 132, in which the results of the treatment of ninety-eight cases are given.

Gonadotropins.—The probability of various chemical constitutions for the gonadotropins, pituitary, serum and chorionic, is borne out by the variation in their physiological actions.

At least nine gonadotropic substances have been postulated, although a number of these may prove to be identical. Further, the follicle-stimulating factors from menopausal urine and from pregnant mare's serum may prove to be identical. Further, this factor (or factors) may be identical with the anterior pituitary F.S.H. It has been suspected that serum gonadotropin is not identical with F.S.H. of the pituitary because the former has given disappointing clinical results in inducing ovulation. If Young's suggestion (*Practitioner*, March 1945, p. 129) is correct, however, this suspicion is founded on a false premis. Young states that the function of pituitary F.S.H. is simply follicle stimulation, and not the induction of ovulation (i.e. *rupture* of the matured follicle). Rupture of the follicle is brought about by pituitary luteinising hormone, and it is therefore not legitimate to expect pituitary F.S.H. or serum gonadotropin to induce ovulation.

The pituitary gonadotropins and the serum and chorionic gonadotropins are of considerable clinical interest and can with advantage be considered together. The postulated gonadotropic substances are :—

From the pituitary :—

1. Follicle-stimulating hormone (Prolan A or F.S.H.)
2. Luteinising hormone (Prolan B or L.H.)
3. Interstitial cell-stimulating hormone (I.C.S.H.)
4. Antagonistic factor
5. Synergistic factor
6. Prolactin

Extra-pituitary substances :—

7. F.S. factor from human menopausal and post-castrational urine

8. Serum gonadotropin (F.S.H.) from pregnant mare's serum

9. Chorionic gonadotropin from human pregnancy urine

Of these, numbers 2, 3 and 4 may be identical, and numbers 7 and 8 may be identical. For the sake of simplicity it is legitimate to assume that this is so. Thus there remains a follicle-stimulating factor and a luteinising factor from the pituitary gland with similar extra-pituitary factors from pregnant mare's serum and from human pregnancy urine respectively, a synergistic factor which appears to enhance the action of these and, finally, prolactin. Prolactin is not generally looked upon as a gonadotropic substance, but it has recently been suggested that it is essential for the initiation and maintenance of secretion of progestin by the corpus luteum. If this is confirmed, prolactin must be regarded as a gonadotropin as well as a galactogogue.

It is chiefly by reason of its gonadotropic hormones that the pituitary has been called the leader of the endocrine orchestra. These substances, so far as is known, act only on the gonads, but their effects are marked and far-reaching, indirectly if not directly. The chemistry of their action is unknown; indeed, it is still debated whether their action is best described as "tropic" (stimulative) or "trophic" (nutritive). Hence the terms "gonadotropin" and "gonadotrophin" are both in common use. Both names are permissible in describing the international standard preparations and in defining the international units of serum and chorionic gonadotropins. It seems to be unlikely, even improbable, that the hormones act as nutrients to the gonads. It is almost certain that they are purely stimulative, and, in this respect at least, are typical hormones, and not nutrients. Thus the terms "gonadotropic" and "gonadotropin" are to be preferred.

The follicle-stimulating gonadotropin (F.S.H.) corresponding to Zondek's prolan A, and resembling serum gonadotropin, acts on the germinal epithelium of the gonads in both sexes. In the female, therefore, it stimulates the development of the primordial follicles and the ovum contained in each.

It has been generally considered that this gonadotropin was

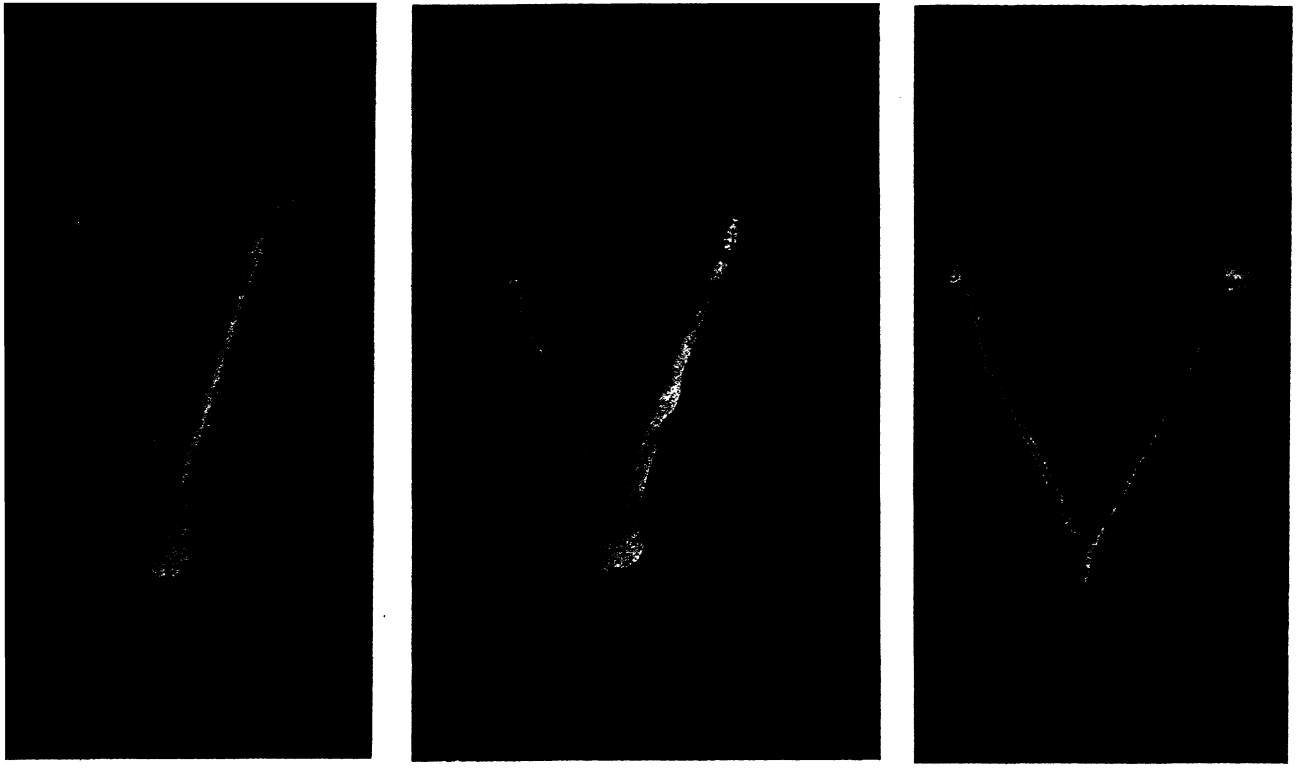


FIG. 3.—GENITAL TRACTS OF TWO IMMATURE (*a and b*) MICE TREATED WITH CHORIONIC GONADOTROPIN (GONAN) SHOWING EFFECT ON GROWTH AND PRODUCTION OF CORPORA HÆMORRHAGICA COMPARED WITH AN UNTREATED CONTROL (c).

responsible for the whole of the maturation process up to and including rupture of the follicle. Some doubt has been expressed, however, as to whether follicle-stimulating gonadotropin does produce rupture of the follicle, and it has been suggested that the luteinising gonadotropin brings about follicular rupture.

The somewhat disappointing results of the clinical use of serum gonadotropin seem to provide some evidence in support of this suggestion, but the rôle of the postulated synergistic factor of the anterior pituitary is not yet clear. It may eventually be shown that the full effect of the follicle-stimulating hormone is not exerted in the absence of the synergistic factor.

It has not yet been shown conclusively whether the follicle-stimulating factor of the pituitary and serum gonadotropin are chemically identical, but for clinical purposes they may be considered to be identical. At the time of writing some pituitary gonadotropins are coming on to the market, but serum and chorionic gonadotropins are in much more general use.

Serum gonadotropin does not stimulate the secretion of testicular hormones in the male, for its action is on the germinal epithelium, whereas the gonadal hormones are secreted by the Leydig cells of the interstitial tissue.

The luteinising factor of the anterior pituitary closely resembles the chorionic gonadotropin. It may be responsible for bringing about the rupture of the mature ovarian follicle, and it is certainly responsible for the formation of the corpus luteum and perhaps for the maintenance of the secretion of progesterone during the second half of each menstrual cycle and during pregnancy.

In the male the luteinising gonadotropin is normally responsible for the development of the testes before puberty and the maintenance of the endocrine function throughout maturity.

It is of interest to note that there is a greater chemical and physiological resemblance between progesterone and testosterone than between œstradiol and testosterone. It is possible, therefore, that the fact that the luteinising factor is necessary to produce the secretion of testosterone and progesterone may indicate some chemical resemblance in the mode of production

of these gonadal hormones under the influence of luteinising gonadotropin.

Clinical Uses of the Gonadotropins

Serum Gonadotropin

In the Female.—When the more obvious symptoms of delayed puberty have been eliminated by treatment with œstrogenic hormone, it may be an advantage to consolidate the results of substitution therapy by giving a course of stimulation therapy with serum gonadotropin. Similarly in other conditions such as amenorrhœa and hypomenorrhœa, serum gonadotropin may often be of value in making the symptomatic improvement produced by œstrogens more permanent.

Serum gonadotropin has been employed for the treatment of sterility attributable to anovular menstruation. Large doses are given in order to bring about full follicular maturation and release of the ovum. Clinical results have been somewhat disappointing when it is used for this purpose. The reason for this is unknown. It may be that the doses employed have been inadequate, serum gonadotropin may not be as active as the similar hormone from the anterior pituitary, or it may be that its full effect is not exerted in the absence of the synergistic factor or of an adequate amount of the luteinising hormone. In an effort to overcome this ineffectiveness, serum gonadotropin has been given intravenously in selected cases, and the results appear to have been somewhat more encouraging.

Serum gonadotropin has been given alone, however, for acne vulgaris *in the female* with satisfactory results. It is not indicated in the acne of adolescence in males.

In the Male.—Defective spermatogenesis is probably the only indication for serum gonadotropin in the male. Treatment should be intensive, and generally vitamin E should be given collaterally. It is possible that, as in the female, somewhat more satisfactory results will be produced if the hormone is given intravenously. Again, however, this route should be used only in selected cases.

Chorionic Gonadotropin

In the Female.—Chorionic gonadotropin is indicated for substitution therapy in conditions arising from deficiency of

luteinising anterior pituitary hormone. The result of such deficiency is imperfect formation of the corpus luteum or of hyposecretion of corpus luteum hormone, progestin. Thus the specific indications for chorionic gonadotropin are the same as those for progestin, except that those conditions in which an immediate response is required must be treated in the first instance with progestin. Thus, spasmodic dysmenorrhœa, acute menorrhagia and threatened abortion should be relieved in the first instance by giving progestin. Thereafter, progestin may be given prophylactically, but this form of treatment must be regarded as a " short-term policy " and when the immediate symptoms have been relieved it is advisable to stimulate the patient's own corpus luteum secretion by giving chorionic gonadotropin. This applies both to pregnant and to non-pregnant patients.

In the female, therefore, chorionic gonadotropin is administered prophylactically in order to prevent menorrhagia, spasmodic dysmenorrhœa and abortion in patients with a previous history of such conditions, or as a means of preventing a relapse in such patients as have been given relief by means of the administration of progestin.

In the Male.—The effect of chorionic gonadotropin in the male is on the purely endocrine interstitial tissues. It has no effect on spermatogenesis. Cryptorchidism, in which the testes fail to descend into the scrotum because of their small size, is thus the first indication for its use. Mechanical obstruction to descent must therefore be eliminated before its use is decided upon. Further, since descent may occur in such cases spontaneously, treatment should generally be withheld until the patient is nine years of age. It is then that the germinal epithelium begins to function, and retention of the testes in the abdomen much after that age may result in damage to the spermatogenic function.

Later in life, subnormal pituitary function may result in deficient testicular function, with consequent gonadal underfunction and sexual under-development. In such cases also chorionic gonadotropin is indicated. On the other hand, when the period of sexual function draws near its end and testicular atrophy and hypoplasia begin, chorionic gonadotropin is contraindicated, for, although it may produce a period of

revived activity, such a period must of necessity be short, and ultimate atrophy will be brought nearer.

Prolactin

Prolactin was so named because of its supposed function of initiating lactation in breasts previously prepared structurally under the influence of the œstrogenic and luteal hormones (gynæcogens) of the ovary. No suggestions appear to have been made as to how prolactin performs this function, and the clinical results of the use of the hormone for deficient lactation have been somewhat disappointing. Probably the precise rôle of prolactin in lactation and its relationship to other lactation-controlling factors remain to be discovered.

It has been reported (*Nature*, Feb. 24, 1940, p. 304) that prolactin is inactivated by phenyl isocyanate. This may indicate that the action of the hormone is enzymatic in nature.

Prolactin possibly has other functions. For example, it has been used in the treatment of menorrhagia and metrorrhagia and stated to have an " antigonadal " effect (*Journ. Clin. Endocrinol.*, 1942, **2**, 296). This is in accord with the suggestion that prolactin is identical with a postulated luteotropic factor secreted by the placenta (*Journ. Amer. Med. Assoc.*, Nov. 18, 1939, p. 192, and *Lancet*, March 27, 1942, p. 387), which is indirectly an antagonist to œstrogen, in that it promotes the secretion of progestin. Further, prolactin is reported to be " uterotrophic ", and thus to be of value in metropathic menorrhagia (*Journ. Clin. Endocrinol.*, Sept. 1944, p. 203 (Supp.)). Perhaps this " uterotrophic " action is merely the result of enhanced progestin secretion, which prolactin appears to produce.

These postulated effects of prolactin on the ovaries and the uterus have not resulted in the general clinical use of the hormone in menstrual derangements. The limited general use of prolactin is almost entirely for the purpose of stimulating lactation in cases in which milk secretion is delayed or insufficient. Results have been variable, and it is doubtful whether the administration of prolactin is in itself sufficient to institute and maintain lactation in most cases in which this function is defective.

In extreme cases anterior pituitary deficiencies may produce

major endocrine disorders, but the most marked abnormalities result from hypersecretion of the various factors, and these are generally the result of pituitary tumours. The cause of these is unknown, and treatment consists in most instances of surgical removal of the tumour.

The clinical uses, doses, and methods of administration of pituitary factors and analogous substances from other sources are most conveniently given in tabular form.

DOSAGE AND TREATMENT WITH PITUITARY AND PITUITARY-LIKE HORMONES.

Condition	Hormone	Dose and Administration	Collateral Treatment and Remarks
Abortion (habitual)	Chorionic gonadotropin	100 I.U. three times weekly for first few weeks of pregnancy, then twice a week. 100 I.U. once weekly from twelfth to twenty-eighth or thirtieth week Intramuscularly	At the most critical periods of pregnancy, and if abortion actually threatens, 2 to 5 I.U. of progestin should be given daily until the danger is averted.
Acne vulgaris	Serum gonadotropin	200 I.U. twice weekly during first two weeks of the intermenstruum. Intramuscularly	Alternatively, œstrogens may be given. An androgen preponderance may be the cause of acne, and œstrogens are suggested in both female and male. Serum gonadotropin is probably not indicated in the male.
Agalactia (mammary hyposecretion)	Prolactin	A series of doses, 300, 300, 120, 120 and 60 I.U. (Riddle-Bates pigeon units) during five days Intramuscularly or deep subcutaneous injection	The hormone of the suprarenal cortex is apparently also essential for lactation and in resistant cases it may be necessary to give suprarenal cortex extract collaterally.
Amenorrhœa (secondary) (and delayed puberty)	Serum gonadotropin	1000 I.U. twice weekly during alternate fortnights Intramuscularly or intravenously (p. 69)	Genital and general development should be made normal and the menstrual function established with œstrogen treatment. Gonadotropin should be given thereafter and administration confined to the first half of the intermenstruum.
Amenorrhœa (primary)	Early cases may be regarded as being examples of delayed puberty (see Amenorrhœa (secondary)). Serum gonadotropin is not indicated in prolonged primary amenorrhœa.		
Anovulation	Serum gonadotropin	1000 I.U. 2 or 3 times in one week	Intravenous injection in dilute solution
Aspermatogenesis, deficient spermatogenesis or spermatozoal amotility	Serum gonadotropin	1000 I.U. weekly, or twice weekly for four or five weeks. Then 200 I.U. once or twice weekly for three or four weeks Intramuscularly, or, in resistant cases, intravenously	Cases with testicular atrophy or eunuchoidism with complete azospermia are not likely to respond to treatment. It may be an advantage to give vitamin E collaterally.

DOSAGE AND TREATMENT WITH PITUITARY AND PITUITARY-LIKE HORMONES—*continued*

Condition	Hormone	Dose and Administration	Collateral Treatment and Remarks
Cryptorchidism	Chorionic gonadotropin	500 I.U. twice weekly for six weeks. In uncomplicated cases weekly injections may be sufficient. Intramuscularly	Mechanical obstruction must be excluded before treatment is undertaken. Up to three courses may be given if necessary, allowing two to four weeks between each course. If this fails, operation should be considered. Treatment may be given to patients of any age *above* nine years.
Delayed puberty	Serum gonadotropin (see Remarks)	1000 I.U. twice weekly. Intramuscularly	Any marked degree of under-development should first be corrected by giving an œstrogen. Treatment should be given during alternate fortnights corresponding as far as possible to first half of intermenstruum.
Dwarfism	Anterior pituitary growth hormone (Phyone)	20 to 50 rat units per dose to a total of 60 to 100 units weekly Intramuscularly	Thyrotrophin, gonadotropins, etc., may be given collaterally as indicated.
Dysmenorrhœa	Chorionic or serum gonadotropin (see Remarks)	Chorionic—500 I.U. twice weekly during *second* half of intermenstruum Serum—200 I.U. twice weekly during *first* half of intermenstruum Intramuscularly	Chorionic gonadotropin in cases with *spastic* dysmenorrhœa after preliminary treatment with progestin Serum gonadotropin if necessary in cases with myohypoplasia after preliminary treatment with an œstrogen
Dystrophia adiposogenitalis (Frölich's syndrome)	Serum and chorionic gonadotropins, growth hormone and thyrotrophin	Any or all of the hormones named may be given, usually in high doses according to the symptoms in each individual case. Intramuscularly	It has been stated that in about 4 per cent. of cases the cause is a pituitary tumour. This must be excluded or, if present, treated surgically.
Endometrial hyperplasia (cystic)	Chorionic gonadotropin (see Remarks)	500 I.U. once or twice weekly during the second half of the intermenstruum during three or four menstrual cycles Intramuscularly	Hæmorrhage must first be controlled by giving progestin (5 I.U. daily) for as long as necessary.
Eunuchoidism.	See Cryptorchidism.		
Frigidity	Serum gonadotropin (see Remarks)	200 I.U. once or twice weekly Intramuscularly	Treatment with gonadotropin is indicated to enhance the effect of preliminary treatment with an œstrogen. Some cases may respond to an androgen, particularly if applied in an ointment to clitoris.
Frölich's syndrome.	See Dystrophia adiposogenitalis.		
Genital hypoplasia (male)	Chorionic gonadotropin	200 I.U. twice weekly. Intramuscularly	Preliminary treatment with testosterone propionate may be given.

DOSAGE AND TREATMENT WITH PITUITARY AND PITUITARY-LIKE HORMONES—*continued*

Condition	Hormone	Dose and Administration	Collateral Treatment and Remarks
Genital hypoplasia (female)	Serum gonadotropin	200 I.U. twice weekly during the first post-menstrual fortnight Intramuscularly	The condition should first be corrected by giving an œstrogen. The gonadotropin is indicated to prevent a relapse.
Hypogonadism. See Genital hypoplasia.			
Hypomenorrhœa	Serum gonadotropin	200 I.U. twice weekly during the first post-menstrual fortnight Intramuscularly	—
Hypothyroidism (with obesity or in Simmonds's disease)	Thyrotrophin	50 guinea-pig weight units two to four times weekly Intramuscularly	Thyrotrophin is not indicated in cretinism or myxœdema in which thyroid hormone should be given.
Impotence	Chorionic gonadotropin	500 I.U. once or twice weekly during intermittent three- or four-weekly periods Intramuscularly	Initial treatment should be with an androgen and if this is successful, chorionic gonadotropin may consolidate the effect if there is no marked testicular atrophy.
Infantilism. See Genital hypoplasia.			
Mammary hyposecretion. See Agalactia.			
Menorrhagia	Chorionic gonadotropin	1500 I.U. in three divided (equal) doses during the ten days immediately before the flow is due Intramuscularly	Before gonadotropic treatment is started, progestin should be given during previous cycles to control the excessive hæmorrhage.
Menstruation (anovular). See Anovulation.			
Metrorrhagia	Chorionic gonadotropin, or prolactin	500 I.U. twice weekly during two or three pre-menstrual fortnights Intramuscularly Or prolactin 200 I.U. daily until hæmorrhage subsides Intramuscularly	This treatment may be given concurrently with progestin during the hæmorrhagic periods to give immediate control of the bleeding. Prolactin alone also gives rapid control.
Obesity.	Thyrotrophin or the gonadotropins. See Dystrophia adiposogenitalis.		
Pituitary dwarfism. See Dwarfism.			
Retained testes. See Cryptorchidism.			
Simmonds's disease. See Hypothyroidism.			
Sterility (female). See Anovulation, Amenorrhœa and Uterine hypoplasia.			
Sterility (male). See Aspermatogenesis.			
Testicular non-descent. See Cryptorchidism.			
Thyroid atrophy. See Hypothyroidism.			
Uterine hypoplasia. See Genital hypoplasia.			

Contraindications for Pituitary Hormones and Chorionic and Serum Gonadotropins

The "tropins" are contraindicated or, perhaps more accurately, *not* indicated in those conditions which superficially may resemble specific deficiency states, but which are ultimately found to be the outcome of deficiencies or unresponsiveness of those glands which are normally stimulated by the "tropins." The principal example of this is probably the thyroid. Thyroid deficiency due to hyposecretion of thyrotrophin is much more uncommon than thyroid deficiency due to iodine deficiency or to inability of the thyroid to respond to pituitary stimulation. Thus most cases of thyroid deficiency cannot be expected to respond to thyrotrophin, and treatment with iodine or with thyroid gland itself is generally indicated.

There are many causes of subnormal growth and of deficient lactation, and such causes should be eliminated before treatment with growth hormone or with prolactin is undertaken. If this is not done there is a considerable risk of administering these hormones unnecessarily and wastefully. Untoward effects are not likely to follow the use of growth hormone given unnecessarily, but in hypothyroid states the administration of thyrotrophin may increase thyroid enlargement if there is no deficient secretion of thyrotrophin by the patient's own pituitary.

No harmful effects are likely to follow the administration of gonadotropins over ordinary periods, with the following exceptions:

1. In the female, serum gonadotropin may cause some derangement of the menstrual cycle if it is given during the *second* half of the intermenstruum.
2. Similarly, chorionic gonadotropin is best administered during the *second* half of the intermenstruum in order to avoid disturbing the menstrual rhythm.
3. In the male, chorionic gonadotropin must not be given for the treatment of cryptorchidism unless mechanical obstruction to testicular descent has been eliminated.

STEROID HORMONES

CHAPTER VIII

OVARIAN HORMONES—THE ŒSTROGENS

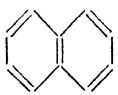
SOME points in the chemistry of the steroids are considered in the chapter on vitamin D (p. 164), but this group of substances is of even greater importance for an understanding of several groups of the hormones, and hence some repetition and further consideration are desirable here. For more detailed information, Feiser's " Chemistry of the Sterids " is still, at the time of writing, the most important book on the subject, although there has been no new edition of this work since 1938.

Hormones which belong to the steroid group include the œstrogens or true ovarian hormones, progestin, the androgens or testicular hormones and the hormones of the suprarenal cortex.

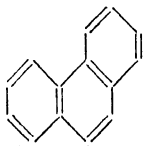
To those who are unfamiliar with it, the basic structure of the steroid nucleus appears to be rather complex, but it is simplified if it is looked upon as being built up from condensed benzene rings.



Benzene.

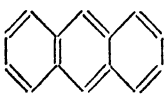


Naphthalene



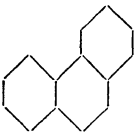
Phenanthrene.

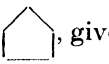
As indicated above, two benzene rings, condensed, can be visualised as forming naphthalene and three as forming phenanthrene. It will be seen that phenanthrene is an isomer of anthracene, the structure of which is represented by the formula

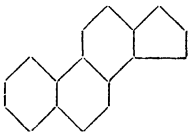


None of the steroids so far discovered or prepared is completely " aromatic " (*i.e.*, possesses the full complement of

double bonds which phenanthrene has), and they are more specifically to be regarded as derivatives of " perhydrophenanthrene ", a fully hydrogenated " condensed cyclic paraffin ".

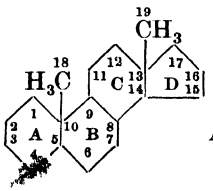


Condensation of this compound with " cyclised pentane " or *cyclo*pentane, , gives *cyclo*pentanoperhydrophenanthrene



A methyl derivative of this is œstrane and the dimethyl derivative is androstane, the parent substance from which progestin, the androgens, suprarenal cortex hormones and cholesterol are derived.

The numbering of this condensed ring system is sufficiently important to give it here (it will be found also on page 166).



Androstane.

Without the methyl group (carbon atom 18) attached to C_{10}, this is œstrane. Androstane plus an ethyl group (C_2H_5— carbon atoms 20 and 21) attached to C_{17} is pregnane and pregnane with its ethyl group oxidised to —CO—CH_2OH is a parent substance of the suprarenal cortex hormones.

It should be noted that the names of all these " parent " substances end with the suffix " -ane ", indicating the saturated or fully hydrogenated compounds—a suffix which they share with the paraffins, methane, ethane, propane, etc.; but most of the physiologically active compounds have one or more unsaturated linkages (double bonds). For these compounds the suffix " -ene " is employed. " -Ene " indicates one double

bond, "-diene" two, "- triene" three, etc. The precise position of these double bonds is indicated by means of the numbers given on the androstane formula above. Œstradiol, the principal ovarian hormone, for example, has three double bonds in ring A, situated between carbon atoms 1 and 2, 3 and 4 and 5 and 6. It is thus an œstratriene derivative. The symbol Δ (capital delta) is employed to indicate double bonds, so that œstradiol is a $\Delta^{1,3,5}$-œstratriene. The termination "-diol" indicates two alcoholic (–OH) groups, and in œstradiol these are attached to carbon atoms 3 and 17. Thus the fully descriptive name for œstradiol is $\Delta^{1,3,5}$-œstratriene-3 : 17-diol or 3 : 17-dihydroxy-$\Delta^{1,3,5}$-œstratriene.

Testosterone, according to the same system of descriptive naming, is 3-keto-17-hydroxy-Δ^4-androstene or Δ^4-androstenedione-3 : 17, and progestin is described by the name 4-keto-Δ^4-pregnene-20-ol or 3-keto-Δ^4-pregnenol-20.

It was once thought that the sterol nucleus was an essential constituent of the molecule of any substance possessing œstrogenic properties, but the discovery of the œstrogenic properties of a group of synthetic substances of which the important members are stilbene derivatives not only threw doubt on this hypothesis, but also caused considerable confusion. A clue which may lead to a solution of the problem has been suggested by Linnell. He has observed that the synthetic œstrogens contain either the *p*-hydroxypropenylphenyl group (*p*-anol), HOCH=CH—CH_3, or some closely related group. The significance of this, however, will be discussed further in a subsequent section on the synthetic œstrogens (p. 104).

The Menstrual Cycle

The œstrogenic hormone of the ovary plays the dominant rôle in the sexual cycles of female mammals. The cycles vary somewhat, but only the cycles in humans will be considered. A general knowledge of the course of the normal menstrual cycle is essential for an understanding of the parts played by the hormones, the nature and ætiology of the pathological conditions resulting from disturbances of the cycle and for a rational application of the hormones in clinical medicine.

Before puberty, sex hormone secretion in male and in female

is minimal, and is insufficient to establish the secondary sex characteristics fully. The final maturation and development of the individual depend on the establishment of the full functioning of the gonads, ovaries or testes. The function of the gonads is under the control of the anterior lobe of the pituitary gland. This in turn is subject to control by the hypothalamus, and perhaps to some extent by some of the cerebral centres. Exactly what brings about the onset of puberty is unknown, but it appears that either the secretion of gonadotropic factors in the pituitary is increased or the gonads become more responsive to these factors. In the female, the consequence of this is a general development of the ovaries.

In the germinal epithelium, primordial folliciles mature under the influence of the follicle-stimulating hormone ("prolan A") from the pituitary. One or more follicles develop, and eventually rupture to release an ovum. The ovary generally, and the developing follicle in particular, secrete increasing amounts of œstradiol, the œstrogenic hormone. It is this hormone which appears to control the development of most of the secondary sex characteristics. The body contours become typically feminine. Fat is deposited in characteristic positions, notably the thighs and buttocks. The breasts develop and the nipples and areoli become pigmented. Body-hair begins to grow, and is confined to characteristic areas—axillæ and pubis. All these changes are not completed in the first menstrual cycle, but may take a few years to reach completion.

When the ovum has been released from the follicle into the peritoneal cavity it finds its way to the ostium of one of the Fallopian tubes and is passed into the tube itself, assisted by the fimbriæ with which the ostium is provided. Then, as a result of the peristaltic action of the tube, it is passed along the tube until it reaches the cavity of the uterus.

One of the effects of œstradiol is to stimulate proliferation of the living cells of the uterine cavity, building up a proliferative endometrium. This process is well advanced by the time ovulation (release of the ovum from the ovarian follicle) takes place. When the follicle has ruptured, it comes under the influence of the second gonadotrpic hormone of the pituitary, the luteinising factor ("prolan B"). This hormone converts the ruptured follicle into a temporary endocrine organ, the

corpus luteum. The corpus luteum secretes the second ovarian hormone, progestin. This second steroid hormone acts upon the proliferated endometrium previously built up under the influence of œstradiol.

The effect of progestin is to elaborate the comparatively simple endometrial structure, producing the secretory phase and maintaining it. In this, the endometrial glands and its histology generally become more complex. The glands become tortuous and the surface becomes rough and pitted. It is then prepared for the reception of the ovum, which by this time will have been passed through the Fallopian tube. The ovum comes to rest on the endometrium, and if it has not been fertilised, it dies in a few hours. The death of the ovum appears to be the signal for degeneration of the corpus luteum. Secretion of progestin rapidly diminishes, and the elaborated structure of the endometrium, the deciduum, begins to disintegrate. The breaking down of the deciduum together with the blood coming away with it constitutes the menstrual or catamenial flow or menstruation.

A normal average menstrual period lasts for four days, although some women normally have a slightly longer period of five, or possibly six days. The whole menstrual cycle occupies twenty-eight days. Women whose menstrual periods are usually longer than the average also tend to have a longer menstrual cycle, and this may be thirty days or as much as thirty-five days.

The rôle and approximate period of action of the hormones in a normal menstrual cycle are illustrated in the diagram on the following page.

Reading from the top of the figure in a clockwise direction, menstruation occupies the first four days of a cycle. Follicle-stimulating hormone secretion is represented at the circumference and the resultant œstradiol secretion is represented immediately inside the F.S.H. During approximately ten days from the end of menstruation the proliferative phase of the uterus (inner circle) is built up. Ovulation follows (F.S.H. effect), and then, during the next three or four days, luteinising pituitary hormone (see outer circle) exerts its effect in forming the corpus luteum and initiating the secretion of progestin (see outer circle). Secretion of progestin results in the develop-

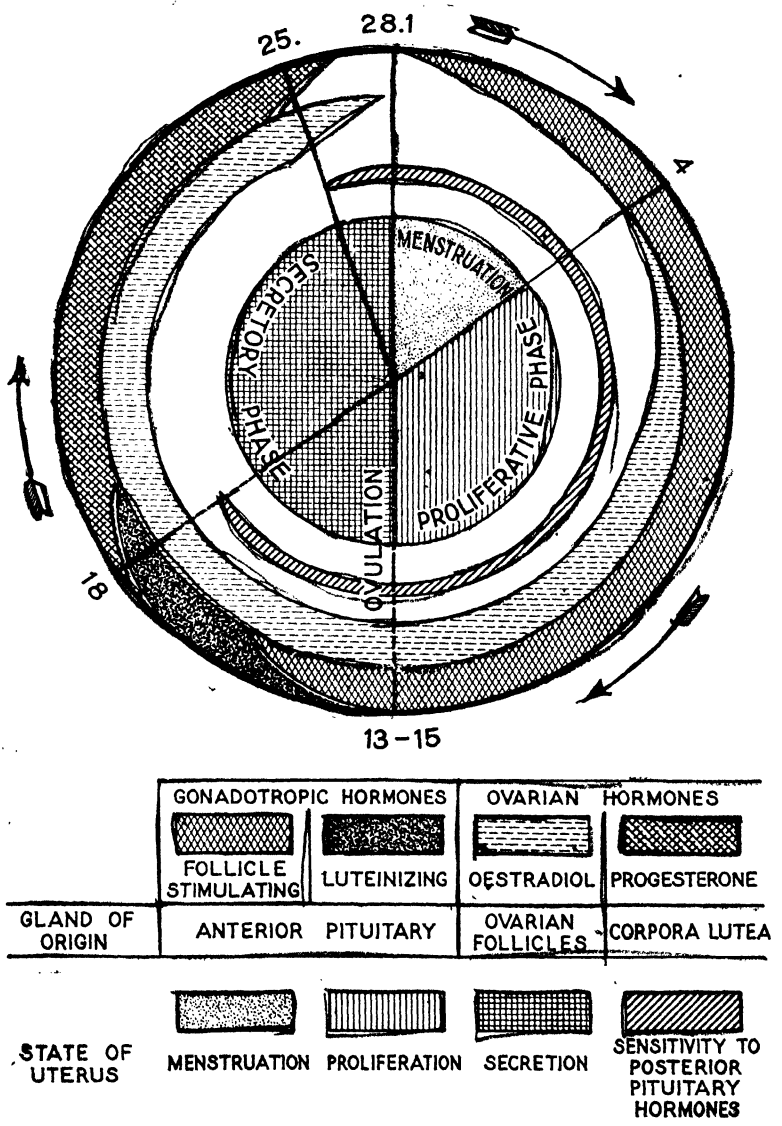


FIG. 4.—DIAGRAM ILLUSTRATING THE RÔLE AND APPROXIMATE PERIOD OF ACTION OF THE HORMONES IN A NORMAL MENSTRUAL CYCLE.

ment of the secretory or progestational phase of the uterus. When secretion of œstradiol and progestin decreases, menstruation follows and another cycle begins. The uterus is susceptible to the influence of posterior pituitary oxytocic hormone during the whole of the cycle, except during that period when the action of progestin predominates over that of œstradiol.

So long as the ovum does not become fertilised this menstrual cycle is repeated regularly from menarche to menopause in a normal woman. In pregnancy, since the ovum does not die, the corpus luteum does not degenerate as in the menstrual cycle. Thus the endometrium does not degenerate, and the ovum is able to maintain its position and to make a secure attachment through the placenta, which begins to develop at this time. By the end of the third month after conception has occurred the placenta should be well developed and functioning as an intermediary between mother and fœtus. In addition to this, the placenta also assumes an endocrine rôle. It begins to secrete progestin by the third month, and the mother's corpus luteum degenerates. Just how many hormones the placenta secretes is unknown, but it does secrete a gonadotropin (chorionic gonadotropin) in addition to progestin, and the gonadotropin is excreted in considerable quantities in the urine, from which it may be isolated for clinical use.

As the end of pregnancy approaches, the secretion of progestin diminishes and the uterus again comes under the dominant influence of the ovarian hormone, œstradiol. This has the effect of sensitising the uterine muscle to the oxytocic hormone of the posterior lobe of the pituitary gland. Thus the onset of labour is facilitated and pregnancy is terminated.

Hormone therapy in the female is intended to correct abnormalities of the menstrual cycle or of the normal course of pregnancy so that a logical and scientific application of the hormones must be based upon a clear conception both of the course of menstrual function and the course of pregnancy and of the precise functions of the hormones in each of these.

Historical Note on the Ovary and its Hormone, Œstradiol

The effects of castration, or ovariectomy, in the female have long been known, although they are not so well or so widely known as the corresponding effects in the male. The

atrophy of uterus and Fallopian tubes and the decline of the secondary sex characteristics in mature females and the non-development of these organs and characters in immature females when the ovaries are removed, point clearly to the existence of an internal secretion. Little or no progress was made in its isolation until Stockard and Papanicolaou showed, in 1917, that characteristic types of cells are produced in the vaginas of guinea-pigs during œstrus, and that these cells can readily be seen in a vaginal smear. Long and Evans confirmed these findings in the rat in 1921, and Allen and Doisy were able to apply these findings and to evolve a method of estimating the potency of ovarian extracts.

By 1923 they had isolated a highly active preparation and other groups of workers also succeeded in this, and the various preparations were named variously, theelin, thelykinin, œstrin and œstrone. Further work showed that several substances had been isolated, chiefly the substances now usually known as œstrone and œstriol. Subsequently it was found that œstrone and œstriol are "excretion" forms of the true ovarian hormone, œstradiol. The establishment of the chemical structure of these substances was followed by their synthesis on a commercial scale. This was of considerable importance in view of the small yields from most of the known sources of the hormone and the consequent high cost of preparations for clinical use. The next step was the discovery of the fact that esters of the hormone had a more intense and more prolonged effect. Of these, œstradiol benzoate has been most widely used and has been adopted as the international standard, with œstrone as the international standard for unesterified preparations.

Comparatively recently, œstradiol dipropionate has been used to an increasing extent and, finally, ethinyl œstradiol is being used in the U.S.A., as it appears to be the most active modification of the hormone when given orally.

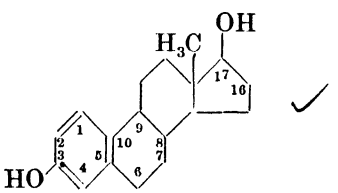
All the natural and synthetic forms of the ovarian hormone still remain too expensive, however, for general clinical use on a really extensive scale. In addition, none of the available preparations is very active when administered by mouth. Thus the synthetic œstrogens, of which the best-known is perhaps stilbœstrol, are used extensively in clinical practice.

A supplementary note on this group of substances, which bear little chemical similarity to the natural ovarian hormone, is appended to this chapter.

Chemistry of the Ovarian Hormone.

It is now generally agreed that the hormone actually produced by the ovary and the ovarian follicles is œstradiol. This has been isolated from the *liquor folliculi* and identified, but the yield is extremely low. Indeed, only 6 milligrams of œstradiol could be obtained from a ton of fresh ovarian substance. Thus the ovary is not capable of storing appreciable amounts of its hormone, and the clinical use of ovarian substance is inevitably disappointing. Nevertheless ovarian substance is still prescribed, although the practice is gradually dying.

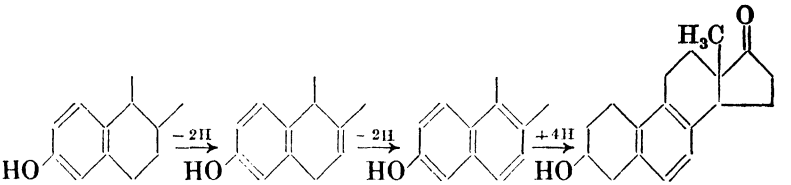
A general reference to the chemistry of the œstrogens has already been made in the beginning of this chapter, where the chemistry of the sterols was briefly surveyed, and it was stated that œstradiol is described more fully by the chemical name, $\Delta^{1,3,5}$-œstratriene-3 : 17-diol. The structural formula of this substance is represented as follows :—



It will be noted that ring A (see page 77) is aromatic (*i.e.*, an unsaturated benzene ring). This is characteristic of all the active natural "excretion" forms of œstradiol; in humans these are chiefly œstrone and œstriol. These last two substances are $\Delta^{1,3,5}$-œstratriene-17-keto-3-ol and $\Delta^{1,3,5}$-œstratriene-3 : 16 : 17-triol, respectively. In mares, some of the œstradiol is oxidised still further to produce equilin ($\Delta^{1,3,5,7}$-œstratetraene-17-keto-3-ol), and equilenin ($\Delta^{1,3,5,6,8}$-œstrapentaene-17-keto-3-ol), both of which substances appear in the urine of stallions as well as in that of mares.

This process of oxidation is not the only chemical change to which the œstrogenic hormone is subjected in natural meta-

bolism. As a further example, it may be noted that $\Delta^{5,7,9}$-œstratriene-17-keto-3-ol has been isolated from the urine of pregnant mares and it has been suggested that this is produced from equilenin by reduction (hydrogenation). The process, starting from œstrone, may be represented by partial formulæ as follows :—



œstrone equilin equilenin $\Delta^{5,7,9}$-œstratriene-17-keto-3-ol

(*Journ. Biol. Chem.*, April 1941, p. 651.)

The process of oxidation of œstradiol to œstrone and œstriol effects a partial inactivation, and so aids in protecting the organism from the effects of " hyperœstrinism ". It is a function of the liver, and synthetic œstrogens appear to be inactivated with varying degrees of efficiency by the same mechanism. Deficiencies of vitamin B_1 or of vitamin B_2 (riboflavine) seriously impair the power of the liver to carry out the inactivating process, and may even deprive it of the power completely. Thus " hyperœstrinism " may be a manifestation of vitamin deficiency in certain cases rather than an indication of ovarian hyperfunction.

A number of isomers of the sterols are possible. For example, if carbon atoms 2, 3, 5, 7, 8 and 10 in rings A and B of the sterol nucleus are visualised as being on the plane of the paper, and the carbon atoms 1, 4, 6 and 9 below that plane (*cis*-decalin compound), the sterol is of the so-called normal or *cis*-type. All the natural sterols, however, are of the *trans*-type. To visualise the spatial relationship of the carbon atoms in this structure, those on the plane of the paper will be numbers 1, 3, 5, 7 and 9, whilst numbers 2, 4, 8 and 10 will be below. Two types of compounds can be formed from these *trans*- (or " allo- ") compounds. If the OH group on carbon atom 3 is below the plane of the paper, the substance is an α (alpha) or *epi-allo*-compound. All the natural sterols belong to the group in which the OH group is on " the plane of the paper ". These

are the *β-allo*-compounds. All the *β-allo*-sterols are precipitated with digitonin, and thus natural compounds can be distinguished and separated from most of the synthetic or artificially modified sterols.

A clear understanding of this much of the chemistry and isomeric structure of the sterols is essential if it is hoped to read some of the extensive literature on steroid chemistry and metabolism with understanding and profit.

Units of the Œstrogenic Hormones

Allen and Doisy introduced a rat unit for œstrogenic substances which was defined as the minimum amount necessary to induce œstrus with complete cornification of the vaginal mucosa, as judged from a smear, in 75 per cent. of a large group of castrated female rats. Subsequently a modified rat unit came into general use, but it has long been realised that these and other biological units are at best only very approximately accurate. Reference to rat units is still found in the literature, however, and one such unit may be assumed to be equal to approximately twenty international units.

The first natural œstrogenic substance to be generally available in the pure state was ketohydroxyœstrin, now generally known as œstrone. This was adopted as the international standard reference standard, and one international unit of this is defined as the œstrogenic activity of 0·0001 mg. (0·1 γ) of this substance. This unit was adopted in 1932. By this time esters of œstrogenic substances were coming into general use, and it was quickly realised that the action of esters (chiefly the monobenzoate) was not accurately expressed in terms of this unit. The esters exert their action over a longer period, and this action is quantitatively greater than that of the unesterified substances, weight for weight. Another international reference standard consisting of an ester was therefore necessary, and in 1935 œstradiol monobenzoate was adopted as the standard preparation. The unit is the international benzoate unit, and is defined as the œstrogenic activity of 0·0001 mg. (0·1 γ) of œstradiol benzoate.

International units and international benzoate units are in common use, but there is a growing tendency to prescribe the œstrogens in terms of weight. Thus the common doses of

œstradiol benzoate may be given as 10,000 to 50,000 I.B.U. or 1 to 5 milligrams.

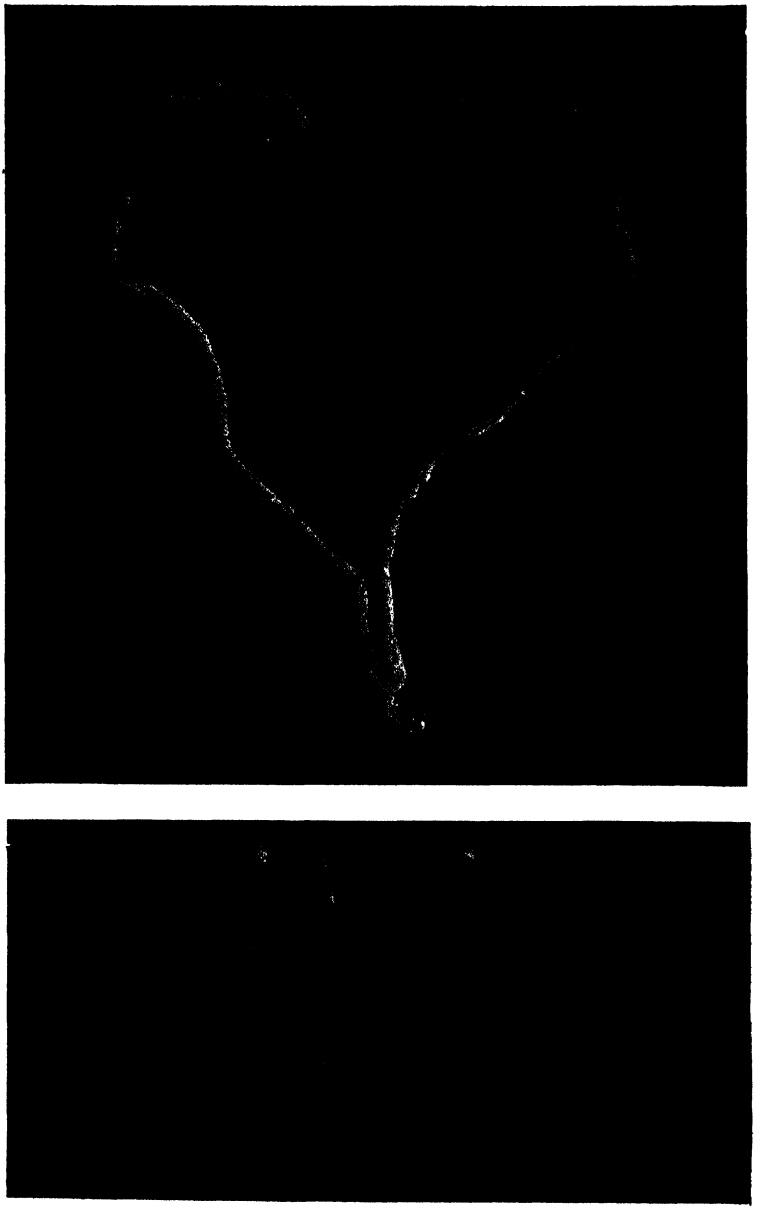
Œstradiol dipropionate is used to some extent, and doses of this are given in terms of weight only. It appears that the dipropionate has a somewhat more prolonged action than the monobenzoate, and therefore the benzoate unit is inappropriate as a means of expressing its activity. For this, as with all other derivatives of the œstrogens, the ready availability of chemically pure substances makes the use of units unnecessary, and œstradiol dipropionate is prescribed in terms of weight only. Similarly with ethinyl œstradiol, which is used to some extent in the U.S.A. and is reported to be the most active preparation of œstradiol when administered orally.

The Physiological Action of Ovarian Hormone

The mode of action of the œstrogens is virtually unknown. It appears that they exert some control over the hydrogen ion concentration of vaginal mucus and the glycogen content of the epithelial cells of the vagina. They exert some control over cellular proliferation and growth, and in this appear to be connected physiologically, and perhaps chemically, with the "organisers" of embryonic growth, differentiation and development.

All body-tissues appear to be affected to some extent by the œstrogens, but the principal effects are on the reproductive system and the secondary sex characters. Thus the full effects of the œstrogens are not seen until after puberty. The development of feminine body-contours, growth of the breasts with development of the duct system, growth of uterus, cornification of vaginal epithelium and development of external genitalia, all result from the increased production of ovarian hormone. Growth of body-hair appears to be stimulated to some extent by œstrogens, but they are probably more concerned in the control of its distribution in the characteristic areas, whereas growth is more specifically controlled by adreno-cortical hormones.

It seems likely that the nasal mucous membrane is to some extent under the control of the œstrogens. Atrophic rhinitis and ozæna respond temporarily to the administration of œstrogens, and it may be that the action in these states is to



a

b

FIG. 5.—GENITAL TRACTS OF IMMATURE FEMALE RABBITS, (*a*) TREATED WITH 150 INTERNATIONAL UNITS OF ŒSTROGENIC HORMONE AND (*b*) AN UNTREATED CONTROL.

some extent a parallel to the similar actions of œstrogens on the endometrium and the mammary duct system.

In large doses the œstrogens are said to inhibit pituitary activity, and this property may be one of the factors essential for the maintenance of the rhythmic repetition of the menstrual cycle.

Œstrogenic Hormone-Deficiency Symptoms

It sometimes happens that the ovaries fail to develop and remain in an " embryonic " state. The effects of such ovarian agenesis may be overlooked in young children, but from the time at which pubertial changes should be appearing the usual signs of sex differentiation fail to appear. General body-growth almost ceases, there is little or no growth of breasts or body-hair and menstruation does not begin. The cause of ovarian agenesis is obscure and there is no known curative treatment. The body-growth and contours may be brought up to normal by the administration of œstrogens, and thereafter the menstrual cycle may be simulated by the combined use of an œstrogen and a " progestogen ". It is of interest to note that no body-hair develops as a result of this treatment, but that the administration of methyl-testosterone collaterally with the female hormone treatment will produce this. Thus androgens seem to control growth of body-hair and œstrogens (or gynæcogens) its distribution (*Journ. Clin. Endocrinol.*, July 1944, p. 317).

All suspected cases of ovarian agenesis should receive treatment with œstrogenic substance, for it is impossible to differentiate this condition from delayed puberty with amenorrhœa (primary), except by direct examination of the ovaries during an exploratory laparotomy. Ovarian agenesis cannot be cured, and normality can only be simulated, but in delayed puberty treatment with an œstrogen appears to assist the ovary in development, as well as to ensure that body-development is normal. The menstrual cycle is established and ovulation may be expected to occur normally.

Secondary amenorrhœa may be the result of a number of different causes. Emotional disturbances sometimes result in secondary amenorrhœa, and the interruption of the menstrual rhythm may be only temporary. On the other hand, in severe

cases secondary psychic effects may cause an indefinitely prolonged suspension of menstruation. Fear that they have lost the normal function may prolong such an amenorrhœic state in some women, and relief will follow the use of œstrogens to produce one artificial menstruation, so demonstrating to the patient that she has not become abnormal. If secondary amenorrhœa has continued for a considerable period, some degree of atrophy may have deprived the uterus of its power of responding to the natural ovarian influence, and it may be necessary to produce three or even four artificial cycles before the natural rhythm is restored. Thereafter normal menstruation may be expected to recur indefinitely at the normal intervals.

Hyposecretion of ovarian hormone may be expected to result in hypomenorrhœa. The volume of the menstrual flow is likely to be subnormal, and it may last for only one or two days or even less. In other cases the flow may appear only at infrequent intervals. It is probable that in hypomenorrhœa the effect of the ovarian secretion is inadequate to build up the normal amount of decidual tissue in the uterus. In oligomenorrhœa, the length of the menstrual cycle is normal, but the volume of flow is reduced.

In some cases of hypomenorrhœa and of otherwise apparently normal menstruation, uterine development as a whole may be subnormal, so that menstruation is taking place from a hypoplastic uterus. This is sometimes a cause of pain, and certain cases of dysmenorrhœa probably fall into this group. Other types of dysmenorrhœa are attributable to excessive uterine motility resulting from corpus luteum hyposecretion, and these will be considered in the chapter on progestin (p. 112).

These quantitative and temporal abnormalities of menstruation constitute the chief indications for the use of œstrogens, with the exception of the climacteric and its associated symptoms. (In order to avoid confusion, the word " menopause " should be understood in its literal meaning of " cessation of menstruation ". The climacteric, in the female, is that period of variable and perhaps considerable length during which the menopause occurs and which includes the pre- and post-menopausal periods of adjustment to a changing endocrine balance.)

Towards the end of reproductive life changes take place in the ovaries as a result of which they appear to lose their power

of responding to the pituitary stimulus. The primordial follicles cease to mature and the secretion of ovarian hormone decreases. In a few cases it appears that there is a sudden increase in the amount of hormone secreted. The cause and significance of this are unknown, but it is probably only a temporary phase.

It is probable that 85 per cent. of women have some symptoms during the climacteric in addition to the cessation of the menstrual function. The symptoms reported are remarkably protean, but the most common is probably flushing of the face and neck. The severity and frequency of the flushes are extremely variable, but in any one patient their frequency is a reliable guide to the progress of the process of adjustment to the new endocrine balance. The flushes can be entirely eliminated by the administration of œstrogens, but in practice it is inadvisable to give doses so large as to produce this effect. Instead, the dose should be sufficient to reduce the incidence of the flushes to a point at which they are not unreasonably severe and to reduce this dose as rapidly as possible without allowing the flushes to become unduly frequent.

Vaginitis is a second relatively frequent symptom in the climacteric. It may be persistent and continue until the other symptoms have disappeared. Most cases of vaginitis must be attributed to œstrogen deficiency, but Shute has reported cases which are aggravated by œstrogens, and therefore seem to be due to an excess of the ovarian hormone. These cases, he reports, respond to vitamin E given in adequate doses (see p. 184).

A more severe degree of atrophy and change occurs in the mucosa and deeper tissues of the vagina, labia minora and vestibule—kraurosis vulvæ, and this, too, generally responds to treatment with œstrogens.

Vaginitis and kraurosis vulvæ are probably direct results of œstrogen deficiency, but it cannot be stated with certainty how such a deficiency causes flushing. One hypothesis is that the diminished ovarian secretion permits an increased secretion of anterior pituitary hormone and this, being a polypeptide, produces a mild " protein " reaction, the chief manifestation of which is the flushing. This theory lacks confirmation, and the real cause of the flushing probably remains to be discovered.

DOSAGE FORMS AND RANGES OF ŒSTROGENS

Œstrogen	Forms	Routes of Administration	Dosage Range
Œstrone and œstradiol	Tablets	Oral	1000 to 10,000 I.U. per tablet.
	Aqueous suspension	Intravenous, subcutaneous or intramuscular	10,000 to 40,000 I.U. (½ to 2 c.c.)
	Suppositories	Per vaginam (adults or children)	1000 I.U. per suppository
	Solution in oil	Nasal instillation (ozæna or atrophic rhinitis)	1000 I.U. per c.c.
	Solution in alcohol	Percutaneous (mammary atrophy or vulvitis)	1000 I.U. per c.c.
	Ointment	Percutaneous (as alcoholic solution)	1000 to 5000 I.U. per grm.
Œstriol	Capsules	Oral	400 I.U. per capsule
Œstradiol monobenzoate	Ampoules of solution in oil	Intramuscular or subcutaneous	1000 to 50,000 I.B.U. per c.c. (0·1 to 5 mg.)
Œstradiol dipropionate	Solution in oil	Intramuscular	1 to 4 mg. (½ to 2 c.c.)
Ethinyl œstradiol	Tablets	Oral	0·02 to 0·15 mg. daily
Stilbœstrol	Tablets	Oral	0·1 to 5 mg. per tablet
	Ampoules of solution in oil	Intramuscular or subcutaneous	1 mg. to 5 mg. per c.c.
Stilbœstrol dipropionate	Ampoules of solution in oil	Intramuscular or subcutaneous	1 to 5 mg. per c.c.
	Tablets	Oral	0·1 to 5 mg. per tablet
Hexœstrol	Tablets	Oral	0·1 to 5 mg.
	Ampoules of solution in oil	Intramuscular or subcutaneous	1 to 5 mg. per c.c.
Dienœstrol	Tablets	Oral	0·1 mg., 0·3 mg., 1 mg. or 5 mg. per tablet

NOTES

1. Ethinyl œstradiol is being used in the U.S.A., but is not yet available in England.

2. Hexœstrol dipropionate has been used parenterally for the inhibition of lactation, and was stated to be better than stilbœstrol or hexœstrol given orally (*Brit. Med. Journ.*, Sept. 30, 1944, p. 428).

3. It is not possible to give exact figures, but œstrone appears to be three to five times as active when given by injection as when given orally. Œstrone in aqueous suspension probably exerts its effect somewhat more rapidly than œstrone in solution in oil when both are given by injection.

4. Ethinyl œstradiol is said to exert a prolonged effect, and therefore to be of special value in controlling menopausal symptoms. The same advantages are claimed for œstradiol dipropionate, given by injection, ethinyl œstradiol being given orally.

5. For convenience in changing over treatment from œstradiol benzoate or œstrone to stilbœstrol or dienœstrol, the following approximate equivalents are given :—

5000 I.B.U. (0·5 mg.) œstradiol benzoate = 1 mg. stilbœstrol
(by injection) = 0·3 mg. dienœstrol (orally)

50,000 I.B.U. (5 mg.) œstradiol benzoate twice weekly
= 1 mg. stilbœstrol three times daily for one week
= 0·3 mg. dienœstrol three times daily for one week

10,000 I.U. œstrone orally = 0·5 mg. stilbœstrol
= 0·2 mg. dienœstrol

1000 I.U. œstrone orally three times daily
= 0·2 mg. stilbœstrol daily
= less than 0·1 mg. dienœstrol daily

6. Triphenylethylene has been employed in laboratory trials as an œstrogen, but is not in common use.

7. Stilbœstrol dipropionate is probably slightly less rapidly active than stilbœstrol itself, and is said to produce less marked reactions such as nausea.

8. Hexœstrol is slightly less active and less toxic than stilbœstrol.

9. The duration of action of œstrogens depends on many factors—method of administration, concentration, speed of absorption and whether esterified or not, and acid with which the œstrogen is esterified. As an approximate guide, the following figures for duration of action may be of value. Equal weights of various œstrogens in solution in oil are injected intramuscularly, and the action lasts as follows :—

Œstrone	4 days
Œstradiol	7 days
Œstradiol benzoate	14 days
Œstradiol dipropionate	42 days

(Dose comparable with therapeutic maximum.)

A few other conditions have been treated successfully with œstrogens. As an example, gonococcal vulvo-vaginitis in children responds to such treatment. The normal vaginal mucosa of a child is not resistant to gonococcal infection, but the cornification brought about by œstrogen therapy successfully overcomes the infection, and no other treatment is generally necessary.

The œstrogenic hormone appears to play some part in the intra-uterine development of infants of both sexes. Premature infants are deprived of this after birth, and development may therefore be subnormal. Administration of the hormone to immature infants is of considerable value in such cases.

At the other extreme of life, obesity may appear in women as part of the climacteric syndrome. Œstrogens have been used in this condition, therefore, but the ætiology of menopausal obesity is obscure and the rationality of the treatment is questionable. It may be that the obesity is a manifestation of thyroid deficiency, short of definite myxœdema, and thus unamenable to treatment with œstrogens.

The Choice of Œstrogen in Therapy

Before considering dosage it is necessary to decide upon the most suitable method of administration in each individual case, and then to choose the most suitable œstrogen. The table on p. 92 with the appended notes gives sufficient information on which to base a preliminary choice, but the final choice will probably be made as a result of clinical experience and the idiosyncracies of individual patients.

Dosage and Administration of the " Natural " Œstrogens

Œstradiol and the related œstrogens, whether prepared from natural sources such as stallion's urine, or the chemically identical substances prepared synthetically, together with their esters, are from the clinical point of view the most satisfactory œstrogens, and may all be included under the heading of the " natural " œstrogens, or œstrogenic steroid hormones. The esters, standardised in terms of international benzoate units and prescribed in terms of these units or by weight (for all are prepared as chemically pure substances), make possible the

closest imitation of the natural secretion of the hormone. In suitable cases this artificial " secretion " can be still further improved and " evened out " by the collateral use of tablets given either orally between injections or by local application of the hormone in the form of vaginal suppositories or ointments.

Further, parenteral administration of esters of œstradiol ensures that dosage is entirely under the control of the physician. This is a point of some importance, as many patients cannot be relied upon to take tablets regularly and at stated times. Some may take more than is necessary, on the principle that twice the dose will do twice as much good as the prescribed dose. Others may forget to take the tablets and then take two or three times the prescribed amount in order to " make up for lost time ".

The following table summarises the principal indications for ' natural ' hormone treatment. The doses given are averages of those which clinical experience has shown to be most generally effective, but it must be understood that it is generally an advantage to vary these for individual patients according to the severity of the condition and the response to treatment. The conditions mentioned are given in groups arranged, in the order of their appearance in the life of the patient.

It is generally recommended that œstrogens should be administered only during the proliferative phase of the menstrual cycle—that is, between menstruation and ovulation, the latter occurring presumably about ten to fourteen days after the former. It is probably advisable to adhere to this as a general rule, but there are exceptions to this. During pregnancy and the puerperium there are of course indications for the use of œstrogens when they cannot be given during the proliferative phase because the menstrual cycle is suspended, and after the menopause the hormone may be given continuously.

Further, it is sometimes permissible to postpone a particular menstrual period once or twice in a patient's lifetime if it would otherwise coincide with a marriage or other more or less unique occasion. This is achieved by administering a œstrogen from a few days before ovulation is expected to take

Condition	Dosage and Scheme of Treatment	Additional Notes on pages
	Prepubertial Disorders	
Prematurity	Premature infants should receive 1000 I.U. of œstrone or œstriol daily in two doses of 500 I.U., dissolved in 1 drachm of warm water.	93
Vulvo-vaginitis in children	One vaginal suppository (1000 I.U.) twice daily and 1000 I.U. orally daily of œstrone or œstradiol, together with the usual routine local and general treatment	94
	Menstrual Disorders	
Amenorrhœa (primary)	Œstradiol benzoate 5 mg. (50,000 I.B.U.) once or twice weekly for two weeks. Alternate fortnights of treatment and rest are continued until menstruation is established. In resistant cases it may be an advantage to give three or four injections of 5 mg. of progestin during the earlier part of the otherwise treatment-free fortnights, allowing five or six days treatment-free at the end of the fortnight.	87 98
Amenorrhœa (secondary)	Œstradiol benzoate 2 mg. (20,000 I.B.U.) twice weekly during alternate fortnights until the cycle is re-established. When it is possible to calculate the phase the cycle would have been in, treatment should be arranged so as to start the cycle at the appropriate time.	87
Arthritis (of endocrine origin—usually menopausal)	Œstradiol benzoate 5 mg. (50,000 I.B.U.) twice weekly until relief is obtained, and then in diminishing doses during six to eight weeks.	89
Dysmenorrhœa (attributable to uterine hypoplasia)	Œstradiol benzoate 2 to 5 mg. (20,000 to 50,000 I.B.U.) twice weekly during the first half of the intermenstruum.	90
Epimenorrhœa (polymenorrhœa)	Œstradiol benzoate 1 to 2 mg. (10,000 to 20,000 I.B.U.) twice weekly for two weeks, followed by progestin 2 I.U. twice weekly during the next two weeks. Repeat during one or two further cycles to establish a normal rhythm.	—

Condition	Dosage and Scheme of Treatment	Additional Notes on pages
Oligomenorrhœa	Œstradiol benzoate 2 mg. (20,000 I.B.U. twice weekly during the first two weeks of each of several menstrual cycles. Larger doses of up to 5 mg. (50,000 I.B.U.) may be required in resistant cases).	—
Delayed puberty	Treatment as for ordinary cases of primary amenorrhœa (*q.v.*)	
	Sterility and Disorders of Pregnancy	
Sterility (due to uterine hypoplasia)	Œstradiol benzoate 5 mg. (50,000 I.B.U.) twice weekly during the two weeks following menstruation during several cycles until uterine development is normal	—
Sterility (due to non-ovulation)	The ideal treatment for this condition has not yet been evolved, but the following is successful in some cases: œstradiol benzoate 10 mg. (100,000 I.B.U.) on the seventh and tenth days after the end of a menstrual period.	—
Simple vomiting of pregnancy	Œstradiol, œstrone or œstriol 10,000 I.U. orally daily until relief is obtained	—
Labour (to induce)	Œstradiol benzoate 2 to 5 mg. (20,000 to 50,000 I.B.U.) four hourly for twenty-four to forty-eight hours	81
Uterine inertia (in the absence of mechanical obstruction)	Œstradiol benzoate 2 mg. (20,000 I.B.U.) hourly for eight to ten hours	81
Inhibition of lactation.	Œstradiol benzoate 5 mg. (50,000 I.B.U.) during the first day of the puerperium and repeated if necessary twenty-four hours later	—
Missed abortion	Œstrodiol benzoate 5 mg. (50,000 I.B.U.) every eight hours until the uterus is evacuated	81
	Menopausal Disorders	
Kraurosis vulvæ	Œstradiol benzoate 10 mg. (100,000 I.B.U.) twice weekly for two or three weeks, followed by 2 mg. (20,000 I.B.U.) for a few weeks. One or two vaginal suppositories of œstrogen should be used daily throughout the period of injections and for a week or two afterwards.	90

Condition	Dosage and Scheme of Treatment	Additional Notes on pages
Artificial menopause	Immediately after surgical or radiological castration, œstradiol benzoate 10 mg. (100,000 I.B.U.) three times a week progressively reduced to 5 mg. (50,000 I.B.U.) twice weekly by the eighth week, and then gradually decreased as in a case of natural menopause	89
Natural menopause	From 1 mg. (10,000 I.B.U.) of œstrone or œstriol orally twice daily in mild cases to 5 mg. (50,000 I.B.U.) of œstradiol benzoate twice weekly, gradually reduced in each instance as rapidly as is compatible with a reasonable control (but not complete elimination) of the symptoms	90
Pruritus vulvæ and Senile vaginitis	Œstradiol benzoate 5 mg. (50,000 I.B.U.) three times a week. The treatment may be supplemented by administration of vaginal pessaries of œstrogen or by the application of œstrogen ointment to the vulva nightly.	90

place and continuing until about five to ten days before a time when it is convenient for the menstrual period to occur. Suitable dosage for this purpose is œstradiol benzoate 5 mg. (50,000 I.B.U.) (intramuscularly) on the first, second, fourth and sixth days, and subsequently every third day for as long as necessary.

Alternatively, stilbœstrol or dienœstrol may be given, 1 mg. of the former or 0·3 mg. of the latter, four times daily for four or five days and three times daily thereafter for as long as is necessary. The effect of this treatment is possibly to inhibit ovulation by inhibiting the secretion of the anterior lobe of the pituitary responsible for ovulation.

Such applications of the ovarian hormone are conformable with the general conception of the menstrual cycle in which the œstrogenic hormone plays a minor or even insignificant rôle in the intermenstruum after ovulation. According to the most recent theory of the menstrual cycle, the secretion of œstrogenic hormone reaches a peak at the time of ovulation, falls slightly, and then rises again to reach a second peak at the

time of maximum corpus luteum activity. The physiological significance of this is not clear, but it is of interest to note that progestin appears to synergise some, if not all, of the actions of the œstrogens. It has been noted, for example, that the excretion level of citric acid is maintained in part by œstrogens. During the post-ovulation phase there may be a drop in the œstrogen secretion to a level below the optimum for the maintenance of normal citric acid excretion, but this is compensated for by the synergistic action of progestin on the œstrogen activity (*Journ. Clin. Endocrin.*, September 1944 (Supplement), p. 225).

A menstrual rhythm can be simulated in patients with no ovaries simply by the administration of an œstrogen, the resultant "menstruation" being an example of "œstrin withdrawal bleeding". A more nearly natural menstrual cycle can be induced by giving progestin for an appropriate time after the œstrogen ("Kauffman technique"). Zondek (1942) found that "progestin withdrawal bleeding" could be induced in amenorrhœic patients, but that results were more satisfactory if an œstrogen and progestin were given simultaneously (see p. 123). More recently still (*Journ. Clin. Endocrin.*, July 1944, p. 317) it has been shown clinically that in sexually infantile amenorrhœic patients sexual development and function can be made almost normal by the following treatment. Development of secondary sex characteristics is brought about by giving stilbœstrol by mouth for as long as necessary. The menstrual cycle is then simulated by giving stilbœstrol 1 mg. daily for fifteen days. For the next six days the stilbœstrol is given as before, but, in addition, ethisterone (anhydrohydroxyprogesterone) is given in doses of 10 mg. daily, and then no treatment for seven days. Menstruation occurs, and the cycle can be repeated indefinitely as long as this scheme of treatment is kept up. Patients treated in this manner remain apparently sexually normal, except that body-hair does not appear and ovulation is not induced. Thus the patient remains sterile in this condition, which is attributed to hypopituitarism. It is of some interest to note that the administration of methyl-testosterone (10 to 25 mg. daily) with the stilbœstrol will induce the growth of body-hair, in the characteristically female distribution.

It will be seen that in certain cases it may not only be permissible, but desirable, to administer an œstrogen collaterally with a progestational substance, particularly if there is reason to suspect that there is some degree of œstrogen deficiency and that the action of the progestin is likely to be enhanced thereby. The details of this combined treatment have not been worked out, and it is therefore not recommended that it should be generally employed clinically.

Thus, to summarise, œstrogens should be administered to menstruating patients only during the first fortnight after a menstrual period, except in those cases in which it is desired to postpone a menstrual period, or when they are given in comparatively small doses collaterally with a progestational substance, progestin or ethisterone.

Some comparison between the dosage of the steroid ("natural") hormones and the synthetic œstrogens has already been given. Further details of dosage of the synthetic œstrogens is given in the chapter on these substances.

Œstrogen Overdosage

Excessive administration of œstrogens, apart from administration at inappropriate times, has not been shown to produce marked undesirable effects, and such effects as are produced disappear when the œstrogen is withdrawn. One effect may be the over-proliferation of the endometrium, with subsequent excessive menstruation. Female secondary sex characteristics may become exaggerated and breasts excessively large. Ovulation may be inhibited or delayed as a result of inhibition of pituitary, secretion and progestin secretion may be reduced in consequence.

Administration of œstrogens continuously or during the second half of the intermenstruum may cause a more or less indefinite postponement of menstruation or a gross overgrowth of the endometrium amounting to a cystic hyperplasia with hæmorrhage. The patient will become normal, however, on discontinuing the œstrogen, and the return to normal may be accelerated by administering progestin.

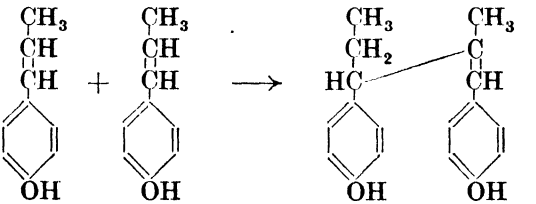
It has been suggested that the use of œstrogens may cause cancer. No convincing evidence that such an effect is ever produced by the therapeutic use of œstrogens is forthcoming.

Œstrogens may act as activators of a cancerous tendency in certain highly susceptible laboratory strains of mice if given over long periods, but in humans doses far in excess of doses used in treatment would have to be given continuously for periods of the order of ten years or more before any comparable effects could be expected. Some clinicians are of the opinion that leukoplakia may be a precancerous condition, so that it may be desirable to administer œstrogens cautiously to patients who have this disease. No case is known, however, in which there is any evidence of leukoplakia being converted into cancer as a result of the use of œstrogens.

Less and less consideration is being given to such suggestions, and there are even claims that the administration of œstrogens exerts a beneficial effect on patients with mammary cancer. This form of treatment needs much fuller investigation, however, before it can be generally recommended.

Supplementary Note

It was once observed accidentally that some unknown œstrogenic substance remained in equine urine after the removal of all œstrogenic steroids. A preliminary examination seemed to indicate that this effect was produced by anol (*p*-hydroxypropenylbenzene), but further investigation by Campbell, Dodds and Lawson (*Nature*, 1938, **141**, 78) indicated that the substance responsible was a " dimer " of anol, dianol.



Anol Dianol

Only minute amounts of dianol are present in urine, but its identity was established and confirmed by synthesis. The œstrogenic properties of dianol suggested that some related substances might be even more active, and Dodds, Goldberg, Lawson and Robinson quickly synthesised stilbœstrol (Nature, 1938, **141**, 247) and demonstrated its activity.

It is accurately described as 4 : 4′-dihydroxy-α : β-diethyl-

stilbene, and Dodds *et al.* suggested the name "diethylstilbœstrol" as a short common name. This name is purely chemical, except for the syllable "œstr", indicating its œstrogenic properties. An entirely chemical name, "diethylstilbenediol", would perhaps have been more appropriate. The use of the syllable "œstr" implies the dihydroxy-diethyl compound, for neither dihydroxystilbene (stilbenediol) nor diethylstilbene has any appreciable œstrogenic properties. As Dodds *et al.* now agree, the œstrogenic compound is more correctly known as stilbœstrol. Diethylstilbœstrol is admitted in the B.P. as a synonym, and in the U.S.A. the Council on Pharmacy and Chemistry of the American Medical Association has laid down that "diethylstilbœstrol" shall be the official name, the name "stilbestrol" being recognised to describe dihydroxystilbene.

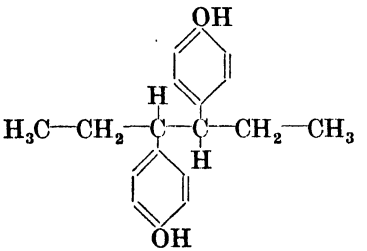
Stilbœstrol occurs in colourless crystals or a crystalline powder, only very slightly soluble in water (only 4 parts per 1000 at 73° F.) but readily soluble in alcohol (95 per cent.) and in ether. It is soluble in oil in any proportions likely to be required pharmaceutically. Stilbœstrol dipropionate is only about one-tenth as soluble in water as stilbœstrol itself.

No special precautions are necessary in storing the synthetic œstrogens. Solutions of stilbœstrol in oil may be sterilised by heating to 150° C. for an hour.

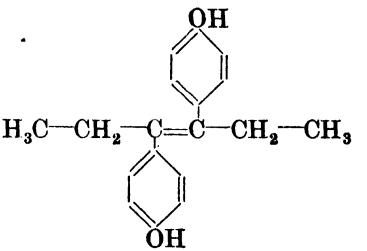
As is usual when a new type of chemical substance with valuable properties is produced, similar substances to stilbœstrol were quickly synthesised and examined with a view to discovering an even more valuable preparation and of determining the precise chemical grouping responsible for the characteristic activity. In the case of the synthetic œstrogens a substance was sought which would be free from the slightly nauseating action of stilbœstrol. Stilbœstrol dipropionate was prepared as well as the "hydrogenated stilbœstrol", hexœstrol. Both these substances came into fairly common use, but a "*de*hydrogenated stilbœstrol" (now called dienœstrol) prepared at about the same time was neglected for several years. It was made available for clinical trial in 1942 following the demonstration by Barnes (*Brit. Med. Journ.*, May 23, 1942, p. 601) of its activity in inhibiting lactation

and in controlling the disturbances accompanying the menopause (*Brit. Med. Journ.*, Jan. 15, 1944, p. 79).

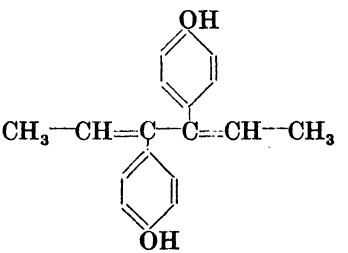
The full chemical names of the three synthetic œstrogens in most general use tend to obscure the fact that they may all be regarded as substitution products of hexane or of oxidised (dehydrogenated) hexane (hexene and hexadiene). The relationship is more clearly indicated by their structural formulæ, which should be compared :—



Hexœstrol (4 : 4′-dihydroxy-γ : δ-diphenyl-*n*-hexane) (dihydrostilbœstrol)

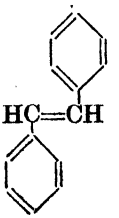


Stilbœstrol (4 : 4′-dihydroxy-α : β-diethylstilbene *or* "4 : 4′-dihydroxy-γ : δ-diphenyl-Δ^3-*n*-hexene")



Dienœstrol ("dehydrostilbœstrol") (4 : 4′-dihydroxy-γ : δ-diphenyl-$\Delta^{3:4}$-*n*-hexadiene)

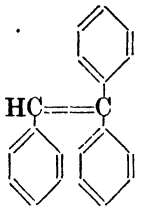
As its generally accepted chemical name indicates, however, stilbœstrol is generally regarded as a derivative of stilbene, the structure of which may be represented thus :—



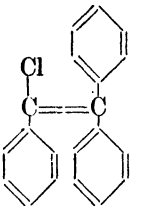
Stilbene itself is not œstrogenic, although it is regarded as a

pro-œstrogen, as are 4-hydroxystilbene and 4 : 4′-dihydroxystilbene.

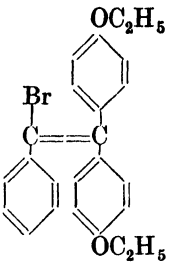
Variations of the stilbene molecule also produce œstrogens which have been used to a limited extent in clinical medicine. Three of these may be mentioned :—



Triphenylethylene

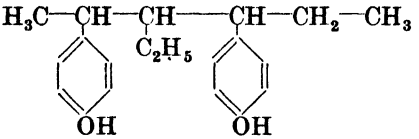


Triphenylchlorethylene



α : α′-Di-(*p*-ethoxyphenyl)-β-phenylbromoethylene—D.B.E.

In the U.S.A. variations on the hexœstrol molecule have been made, and one such compound was introduced under the proprietary name " Octofollin ". This name has been abandoned, and the substance is now known by the non-proprietary name benzestrol. Its chemical name is 2 : 4-di-(*p*-hydroxyphenyl)-3-ethylhexane, and its structure is therefore :—



—a substitution product of hexane.

Mode of Action of Synthetic Œstrogens

It may be assumed that some as yet unidentified molecular formation common to both natural and synthetic œstrogens is responsible for their specific activity. The *trans* isomers only of the synthetic œstrogens are active, and it has been suggested that the general molecular shape of these compounds resembles that of œstradiol and the steroid œstrogens, and that this is a factor in determining their action. This hypothesis is not now generally accepted. It seems more likely that Linnell's suggestion of the significance of anol (*p*-hydroxypropenylphenol) is of more importance.

Indications and Method of Administration of the Synthetic Œstrogens

Most of the synthetic œstrogens have been administered intramuscularly in solution in oil, but their principal advantage over the natural or steroid œstrogens is their high activity when they are given by mouth. Intramuscular injection has been suggested for patients who experience nausea or vomiting following oral administration, but in such cases it is generally preferable to give a steroid œstrogen parenterally.

The oral route is therefore to be regarded as the principal one for the administration of the synthetic œstrogens.

In some instances, more particularly when an intense general systemic action is not required, but when a marked local effect is desired, the synthetic œstrogens may be administered percutaneously in the form of ointments, generally in a " vanishing-cream " base. This method is most useful in pruritus or kraurosis vulvæ, or in some cases of mammary hypoplasia. Œstrogens, natural or synthetic, given by this or any other method, are not likely to be of value in " rejuvenating " the skin or removing lines and wrinkles.

The indications for the synthetic œstrogens are precisely the same as those for the steroid œstrogens, and for details reference should be made to the appropriate section of the chapter on the ovarian œstrogenic hormones (pp. 89 *et seq.*).

Dosage of Synthetic Œstrogens

In the following table suggested doses are given only for the most important conditions. These may be varied for individual patients at the discretion of the physician.

The pharmacopœial dose of stilbœstrol (B.P. 6th Addendum) is 0·5 mg. to 2 mg., but in practice the dosage covers a considerably wider range.

The synthetic œstrogens are not standardised in terms of international or biological units. They are all chemically pure substances, so that doses are expressed in terms of weight only. It is desirable, however, to have some guide to their approximate activity in terms of units, so that if a physician decides to give a patient stilbœstrol, for example after treatment has been given with a steroid œstrogen (or vice versa), he may be able to estimate what dose is required.

DOSAGE OF SYNTHETIC ŒSTROGENS.

Condition	Dienœstrol	Hexœstrol	Stilbœstrol*	Stilbœstrol dipropionate†	Triphenyl-chloroethylene‡
Amenorrhœa, secondary	0·3 mg. three times daily during fortnightly periods corresponding to first half of intermenstruum	Dosage and administration precisely as for stilbœstrol	1 mg. three times daily, orally during fortnightly periods corresponding to first half of intermenstruum	5 mg. two or three times weekly, by injection, during fortnightly periods corresponding to first half of intermenstruum	400 mg. to 600 mg. orally daily during fortnightly periods corresponding to first half of intermenstruum.
Dysmenorrhœa, due to myohypoplasia	0·3 mg. two or three times daily during first half of intermenstruum		1 mg. once or twice daily during first half of intermenstruum	5 mg. weekly, by injection, during first half of intermenstruum	250 mg. intramuscularly immediately after and one week after cessation of menstrual periods.
Kraurosis vulvæ	0·6 mg. once or twice daily		2 mg. three or four times daily	10 mg. two or three weekly	400 to 600 mg. daily
Lactation, inhibition of	0·6 mg. two or three times daily for two days and then daily		1 mg. three or four times daily until the flow has ceased	—	—
Menopause and climacteric	0·1 mg. to 0·6 mg. daily as necessary to control the symptoms		1 mg. once to thrice daily as necessary to control symptoms	5 mg. one to three times weekly, decreasing as rapidly as possible	Initial doses of 200 mg. to 600 mg. three times daily (orally), then decreasing. By injection, 250 mg. weekly, then once every 3 or 4 weeks.
Oligomenorrhœa	0·3 mg. once or twice daily during first half of intermenstruum		1 mg. once or twice daily during first half of intermenstruum	5 mg. once or twice weekly during first half of intermenstruum	200 mg. to 400 mg. three times daily (orally) during first half of intermenstruum.
Pruritus vulvæ and senile vaginitis	0·3 mg. three or four times daily until some relief is obtained, then 0·3 mg. once daily		1 mg. three or four times daily until some relief is obtained, then 1 mg. daily	5 mg. three or four times weekly until relief is obtained, then 5 mg. once weekly	200 mg. to 400 mg. two or three times daily, decreasing to 200 mg. on alternate days (orally).
Sterility, due to uterine hypoplasia	0·3 mg. once or twice daily during first half of intermenstruum		1 mg. twice or thrice daily during first half of intermenstruum	5 mg. two or three times weekly during first half of intermenstruum	—
Prostatic carcinoma	5 mg. daily in divided doses of 1 mg. or 2 mg., or not less than 0·3 mg. daily, preferably in divided doses of 0·1 mg.		5 mg. three or four times daily, or 1 mg. three times daily for two or three weeks, 1 mg. twice daily for three or four weeks, then 1 mg. daily indefinitely	May be employed orally in the same doses as stilbœstrol itself. Parenteral administration is unnecessary.	—

* Stilbœstrol may also be given intramuscularly, but it is usual to employ the dipropionate when the parenteral route is decided upon.
† Stilbœstrol dipropionate may be given orally in the same doses as stilbœstrol itself, but it is more commonly given by intramuscular injection as indicated above.
‡ Orally the duration of action of triphenylchloroethylene is comparable with that of stilbœstrol, but by injection it is remarkably prolonged. The latter method of administration is best reserved therefore for menopausal patients.

Similarly the relative potencies of the synthetic œstrogens are not known accurately, but approximate figures are required for similar reasons.

In using the figures in the following table it must always be remembered that they are by no means mathematically correct; and when changes are made the physician may find it necessary to make adjustments. Further, the esters and triphenylchloroethylene have a more prolonged, and therefore less marked immediate effect than unesterified stilbœstrol, hexœstrol and dienœstrol, but the effect of the esters is probably greater over a period than the simple substances.

Contraindications to and Effects of Overdosage of Synthetic Œstrogens

The contraindications to the synthetic œstrogens are essentially the same as those to the natural œstrogens—that is, they should generally not be given during the second half of the intermenstruum in patients who are still menstruating, except with the deliberate intention of postponing a menstrual period or to lengthen abnormally short cycles. Patients with cancer, or who are suspected of having cancerous tendencies, should not be given œstrogens except in cases of cancer of the prostate and possibly mammary cancer. Cancer of the uterus seems almost certainly to be an absolute contraindication to the use of œstrogens.

The synthetic œstrogens so far introduced may be slightly more carcinogenic than the steroid hormones, but the difference is probably so little as to be of no significance in clinical medicine. In the absence of an abnormal carcinomatous tendency none of the synthetic œstrogens will exhibit carcinogenic properties unless they are given uninterruptedly for excessively long periods and in doses far in excess of those suggested for use in clinical medicine. Even so, it is doubtful whether the term carcinogen is truly applicable to the œstrogens, for they evocate rather than engender cancer. "Chemical and physical carcinogens are provocative only in that they provoke malignant potentiality into activity and their rôle is then ended. They do not increase in quantity as the growth enlarges and they have no effect in determining the kind of tumour which arises" (*Nature*, Nov. 20, 1943, p. 584).

COMPARABLE DOSES OF STEROID AND SYNTHETIC ŒSTROGENS

Œstrone	Œstradiol benzoate	Dienœstrol	Hexœstrol	Stilbœstrol	Stilbœstrol dipropionate	Triphenyl-chloroethylene
1000 I.U. (0·1 mg.) orally	Not given orally	—	0·066 mg. orally	0·066 mg. orally	0·06 mg. orally	2·5 mg. orally
1000 I.U. (0·1 mg.) parenterally	<1000 I.B.U. (0·1 mg.) parenterally	0·1 mg. orally or parenterally	0·33 mg. orally or parenterally	0·33 mg. orally or parenterally	0·3 mg. orally or parenterally	13 mg. orally
10,000 I.U. (1 mg.) orally	Not given orally	0·2 mg. orally or parenterally	0·6 mg. orally or parenterally	0·6 mg. orally or parenterally	0·5 mg. orally or parenterally	20 mg. orally
10,000 I.U. (1 mg.) parenterally	<10,000I.B.U. (1 mg.)	1 mg. orally or parenterally	3 mg. orally or parenterally	3 mg. orally or parenterally	2·5 mg. orally or parenterally	120 mg. orally
	5000 I.B.U. (0·5 mg.) parenterally	0·5 mg. orally or parenterally	1·25 mg. orally or parenterally	1·25 mg. orally or parenterally	1·25 mg. orally or parenterally	40 mg. orally
	50,000 I.B.U. (5 mg.) parenterally	3 mg. in divided doses daily over 3 days or 0·3 mg. thrice daily	10 mg. in divided doses over 3 or 4 days or 1 mg. thrice daily	10 mg. in divided doses over 3 or 4 days or 1 mg. thrice daily	10 mg. parenterally	400 mg. orally

Thus the usual contraindications to the œstrogens are those conditions attributable to hypersecretion of ovarian hormone or to hyposecretion of corpus luteum hormone, in particular menorrhagia and metropathia hæmorrhagica, as well as uterine fibromyomata and cystic hyperplasia of the endometrium.

Excessive or excessively prolonged administration of œstrogens may give rise to these hyperœstrogenic manifestations, but the symptoms will subside on discontinuing the administration when the endocrine balance has been restored.

In menopausal patients it is important not to give excessively large doses, and the amounts given must be gradually decreased, otherwise the endocrine imbalance will be prolonged indefinitely.

Œstrogens in the Treatment of Cancer

It was suggested in 1941 in the U.S.A. that stilbœstrol is of value in controlling or even diminishing the symptoms in prostatic carcinoma. Dodds, in England, has confirmed this, but the precise mode of action, the most suitable dosage and the relative merits of œstrogen therapy and orchidectomy have not yet been finally agreed upon. It is evidently important to avoid inadequate dosage, for small doses of œstrogens are said to potentiate the action of androgens, and thus to aggravate prostatic carcinoma (*Lancet*, Feb. 27, 1943, p. 276). Further, it is most important to exclude benign prostatic hypertrophy, which is thought to be a result of diminished androgen secretion and consequent œstrogen preponderance.

Prostatic carcinoma having been diagnosed and confirmed, œstrogen therapy (using either stilbœstrol or dienœstrol) may be decided upon. Estimation of serum acid phosphatase should be included in the procedure in confirming or excluding metastases to prostatic carcinoma, and estimations during treatment provide useful information as to the efficacy of treatment. A suitable method of estimation is Gutman and Gutman's modification for acid phosphatase of King and Armstrong's method for alkaline phosphatase as described in the *Journ. Biol. Chem.*, **136**, 201 (Oct.) 1940. Acid phosphatase is secreted by the acinar epithelium of the prostate. Traces may be detected in the serum of infants, and the level increases with age. It remains normal in prostatic cancer,

while the lesion is confined to the capsule, but it is increased when metastases appear, especially skeletal metastases. The relevant phosphatase levels have been given in the *Journ. Amer. Med. Assoc.*, Jan. 6, 1945, p. 17, and are as follows :—

	King-Armstrong units.	Bodansky units.
Normal	4·5	0·7
Pathognomic of metastases to prostatic carcinoma . . .	10	>1·0

The object of treatment with œstrogens is to produce an œstrogen preponderance which appears to be inimical to development of the malignant neoplasm. A similar effect is produced by orchidectomy, and in certain cases the two forms of treatment can perhaps be combined advantageously. The position is not fully understood, however, and the interrelationships of various factors must be investigated in greater detail. For example, orchidectomy results in hyperactivity of the anterior pituitary, with consequent increase in the secretion of adrenal androgens. Thus œstrogen treatment is said to be preferable to orchidectomy. Dosage of œstrogens must be adequate, for it has been suggested that low dosage of œstrogens results in a potentiation of androgen activity.

On the whole it may prove that œstrogen therapy is the most desirable form of treatment, for, unlike orchidectomy, it is not followed by a rise in the secretion of gonadotropins and androgens and the serum acid phosphatase, after a preliminary fall, rises more slowly in œstrogen treatment than after orchidectomy (*Surgery*, Aug. 1944, p. 169, abstracted in *Journ. Amer. Med. Assoc.*, Nov. 4, 1944, p. 665).

Conflicting reports have appeared of the value of œstrogens in mammary carcinoma, and in view of this and of the fact that no rational explanation of any beneficial effect they may have has so far been put forward, it is suggested that the treatment should not be employed clinically until more satisfactory experimental evidence of value is produced.

The higher ranges of doses of œstrogens given in the table on page 106 for the treatment of prostatic carcinoma are probably the most suitable. The risk of androgen potentiation

is avoided with these higher doses, and they may be reduced subsequently in accordance with the clinical condition of the patient. Further, the lower doses are frequently not sufficiently low to avoid some degree of gynæcomastia. However, the principal discomfort of gynæcomastia can be avoided by the use of a belt instead of braces, although the subjective embarrassment is perhaps not quite so easily avoided!

Stilbœstrol has hitherto been most extensively used in prostatic carcinoma, but it may cause some degree of nausea and vomiting in a proportion of patients. For this reason dienœstrol is perhaps to be preferred, no such reactions having been reported following its use.

CHAPTER IX

OVARIAN HORMONES—THE PROGESTOGENS

AFTER the rupture of ovarian follicle and release of the ovum in mammals, the ruptured follicle is converted into an endocrine gland, the corpus luteum.

The importance of the corpus luteum was realised by Born, an embryologist who postulated that the gland had a function in pregnancy, observing that it occurs only in mammals and that it reaches maximum development only during pregnancy. Confirmation of Born's postulate was provided in 1903 by Fraenkel, who showed that the embryos disappear in rabbits if the corpora lutea are removed during the first few days of pregnancy.

The histological effect of the corpus luteum upon the endometrium was discovered in 1907 by Loeb, working with guinea-pigs, and this was investigated in more detail in rabbits by Bouin and Ancel in 1910. Then followed attempts to isolate the substance in corpora lutea, which is responsible for these endometrial changes. Hermann, in 1915, showed that the hormone was fat-soluble, but he did not differentiate between the growth of the uterus and the effect on the endometrium. Thus he had not separated the luteal hormone from the œstrogens. Some confusion resulted from this, and further progress was delayed until Corner, in 1928, found that corpus luteum extracts produced endometrial progestational proliferation and ovarian extracts produced uterine growth. Thus the way was opened for the isolation of the hormone; it was discovered that it could only act on a uterus previously sensitised with an œstrogen and a method of standardisation became possible.

Crystalline substances with luteal activity were isolated during 1931 and 1932, but it seems to be unlikely that the pure hormone was isolated before 1934.

Two crystalline substances were isolated which had different melting points. The activity of both was found to be the same and the difference in melting points was found to be due to

variations in the conditions of crystallisation. The two varieties are known as α- and β-progesterone.

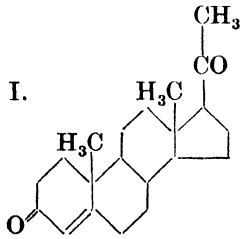
Marrian, in 1929, had isolated an inactive alcoholic substance from the urine of pregnant women which Butenandt in 1930 found was pregnanediol. Subsequently Butenandt showed that pregnanediol could be converted into progesterone and it is now agreed that pregnanediol is the excretion form of progesterone, being excreted as the glucuronide (Venning and Browne, 1936). It is remarkable that progesterone is excreted in this form by women and by normal or castrate men following its injection, but no other animals excrete pregnanediol or its glucuronide.

Chemistry of Progestin

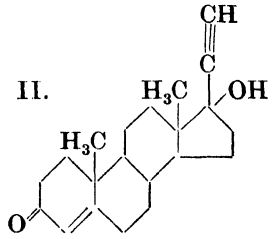
Progestin has already been mentioned in the previous chapter as a derivative of pregnane and androstane. Reference should be made to this chapter and to the section of the chapter on vitamin D dealing with the chemistry of steroids.

Progestin or, officially and more correctly, progesterone (B.P. 7th Addendum), is a diketo-compound with the structure represented by the formula I below. Formula II, shown for comparison, is the synthetic substance, active orally, and known variously as ethisterone, anhydrohydroxyprogesterone, pregneninolone, ethinyl-testosterone and under the proprietary names " Progestoral " and " Proluton C " (now " Oraluton ").

I.



II.



Progestin (I) is Δ^4-pregnene-3 : 20-diketo, or, as given in the seventh addendum to the B.P., 3 : 20-diketo-Δ^4-pregnene. Ethisterone is 3-keto-17-ethinylhydroxy-Δ^4-androstene.

Progestin and ethisterone are both stable substances, and in the forms in which they are issued for clinical use they do not appreciably decrease in activity over periods of two years or more. Progesterone is inactive when administered orally,

apparently because it is inactivated by the digestive enzymes. Ethisterone is more stable and has been introduced for oral administration when injection is objected to, or as a means of augmenting the effect of progestin itself.

Progestin is invariably administered by the intramuscular route.

Units of Progestin

The first estimations of potency of corpus luteum extracts were based upon the progestational effects on the uteri of rabbits previously treated with an œstrogen. After the preparation of chemically pure progestin, which was adopted as the international standard of reference, accurate standardisation became possible, an international unit could be precisely defined and accurate dosage was assured. The rabbit units, as defined by Corner and Allen and by other investigators, were variable because no invariable reference standard was available with which they could be compared. When the pure hormone became available, however, a unit could be agreed upon which was the progestational activity of a definite weight of pure hormone. This was done, and an international unit is the activity of 1 milligram of pure progestin. The pure natural hormone was eventually prepared on a commercial scale from the corpora lutea of whales, but now most of the hormone is prepared by synthesis from stigmasterol or some other suitable sterol. Thus the need for a unit is practically non-existent, and there is an increasing tendency to define the potency and prescribe the hormone in terms of weight of pure hormone, one milligram of which has an activity of one international unit.

Ethisterone was introduced into clinical medicine as a pure chemical substance of known chemical constitution, so that it has been unnecessary to express its potency in terms of a unit, as was necessary with progestin. Dosage of ethisterone is thus expressed in terms of weight only. The oral dose of ethisterone in milligrams is approximately six times greater than that of progestin given intramuscularly.

Estimation of Progestin

Progestin and preparations containing it are estimated by comparison with the international standard preparation of

the pure hormone by a biological method, the pregravid changes produced in the uterine horn of immature female rabbits being the criterion of activity.

Physiological Action of Progestin and Ethisterone

The mode of action of the progestogens, like that of the other steroid hormones, is unknown. They act almost entirely on tissues previously acted upon by œstrogens, bringing about an elaboration of these tissues, particularly the endometrium and mammary tissues. The effect on the endometrium has already been described in the previous chapter. The effect on the breasts is analogous in that it consists of elaboration of tissue previously built up under the influence of œstrogens. Ducts and acini are developed to the stage at which milk secretion becomes possible if these prepared tissues come under the influence of prolactin, the galactotropic hormone of the anterior lobe of the pituitary gland.

The progestogens have properties which appear to indicate that they are in a sense physiological antagonists to the œstrogens. First, and most important, is the antihæmorrhagic effect on the uterus in menorrhagic states whereby uterine hæmorrhage is controlled. A second property of progestin may also have some subsidiary effect in controlling hæmorrhage, the antiœstrogenic effect whereby uterine motility is inhibited. This effect consists in desensitising the uterine muscle (myometrium) to the oxytocic effect of posterior pituitary hormone. The same property of progestin is utilised in the treatment of spastic dysmenorrhœa, in which condition the uterine muscle is unduly responsive to the oxytocic hormone and goes into painful spasmodic contraction. Similarly, progestin may be employed to mitigate tonic contraction of the uterus during labour and to relieve after-pains following childbirth.

During pregnancy, progestin is indicated to maintain a "progestational" endometrium, and so to provide for the retention and satisfactory nidation of the fertilised ovum and the secure attachment of the developing fœtus, especially during the first trimester before the placenta has become fully formed and functional.

The fully functioning placenta secretes progestin and probably other hormones also, but if it should be hypofunctional

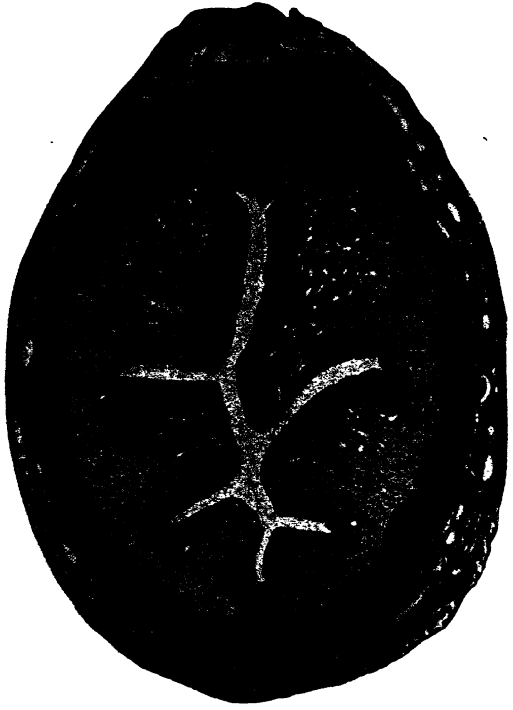


FIG. 6.—RABBIT UTERUS (SECTION) TREATED WITH ŒSTROGENIC HORMONE (ŒSTROFORM) (PROLIFERATIVE PHASE).



FIG. 7.—SIMILARLY TREATED RABBIT UTERUS AFTER TREATMENT WITH ŒSTROGENIC HORMONE FOLLOWED BY 1 UNIT OF PROGESTIN.



FIG. 8.—RABBIT UTERUS TREATED WITH ŒSTROGENIC HORMONE FOLLOWED BY 1½ UNITS OF PROGESTIN (PROGESTATIONAL PHASE).

in this respect, the administration of progestin is again indicated, in threatened abortion in large doses.

Menstruation is inhibited from an otherwise normal endometrium either by administration of an œstrogen or of progestin for as long as either hormone is administered in suitable doses. When administration of either hormone is discontinued, the endometrium begins to disintegrate and œstrin- (or progestin-) withdrawal bleeding occurs a few days later.

Finally, progestin acts synergistically with œstrogens as has been indicated in the previous chapter.

Ethisterone acts in an essentially similar manner to progestin, but, unlike the natural hormone, it has the property of acting when administered orally. There has been a single report to the effect that when ethisterone has been administered for a relatively short time, the body loses its power of responding and that progestin must then be administered (*Proc. Roy. Soc. Med.*, Oct. 1944, p. 677), but this observation is unconfirmed, and does not appear to be in accord with general clinical experience.

Clinical Uses of Progestin and Ethisterone

The progestogens are indicated generally only for the treatment of specific abnormalities which occur only during the reproductive period of life in the female. Œstrogens are sometimes indicated during infancy, and before the normal onset of menstruation. There are no indications for progestin in prepubertial patients. Similarly, œstrogen treatment is specific for many abnormalities of the climacteric, but progestogens are not generally recommended during the climacteric. They produce an endometrial structure from which hæmorrhage takes place, so that they tend to produce an apparent revival of the menstrual function, although they may temporarily control menopausal metrorrhagia. Thus the progestogens are indicated for the control of abnormalities of menstruation and pregnancy.

In dysmenorrhœa of the spastic type progestin is administered in order to inhibit uterine motility.

Excessive and prolonged menstruation (menorrhagia) is an indication of progestin deficiency and consequent œstrogen excess. Progestin, administered in suitable doses at the

appropriate time, is therefore specific in this condition, although it is necessary to exclude organic causes of the menorrhagia such as fibroids. However, even if uterine fibroids are present, progestin may be of some value in the early stages, in that it seems to have some antifibrogenic properties.

Functional uterine hæmorrhage of two types has been described, ovular and anovular. In the ovular type it is probable that there is a transient lowering of the hormonal level during the interval between the rupture of the ovarian follicle and the formation of the corpus luteum. This would account for some degree of endometrial disintegration with consequent hæmorrhage. The condition is probably related to " mittelschmerz " and ovular bleeding. Progestin will prevent this hæmorrhage. In anovular hæmorrhage atresia of the ovarian follicle, with consequent non-formation of corpus luteum, is the cause of prolonged œstrin secretion, uncontrolled by subsequent progestin action. The sequel of this is cystic glandular hyperplasia of the endometrium. Hæmorrhage may take place at any time from a hypertrophied endometrium of this type, but progestin will control it by producing a secretory or progestational endometrium which will disintegrate normally when treatment is discontinued after being given for a sufficient period. The administration of progestin for anovular uterine hæmorrhage is therefore only palliative substitutional treatment. Really curative treatment must consist of some means of ensuring that ovulation is brought about, and that this is followed by adequate corpus luteum formation and function. This has been attempted by giving large " shock " doses of œstrogen and by administering gonadotropins.

Ethisterone is indicated for the treatment of the milder and less urgent conditions in which progestin is employed and as a means of augmenting the effects of progestin in any of its indications. The one exception to this is the treatment of threatened abortion. In this condition, progestin in large doses is indicated, together with complete rest and the other measures generally employed.

Dosage of Progestin and Ethisterone

Dysmenorrhœa.—2 mg. (2 international units) of progestin three days before the expected onset of menstruation and 2 mg.

one day before the expected onset. If this does not give sufficient relief, the doses should be increased to 5 mg. each before the subsequent period. The treatment should be repeated in several cycles until the polarity of the uterus is normal and menstruation is painless without treatment.

Ethisterone, 5 mg. daily, may be given for the same period as progestin is being injected. Mild cases of dysmenorrhœa may respond to ethisterone only, given in doses of 10 mg. daily for three or four days before the expected onset of menstruation.

Threatened Abortion.—5 to 10 mg. (5 to 10 international units) of progestin as soon as the condition is recognised. This dose should be repeated in a few hours if necessary, and then 2 mg. should be given daily until the patient has been free from symptoms for several days. Thereafter, 2 mg. of progestin should be given weekly until the thirty-second week of pregnancy.

Habitual Abortion.—2 mg. of progestin weekly until the thirty-second week of pregnancy. At the times when menstruation would have appeared had the patient not become pregnant, abortion is most likely, and the dose should be increased to 5 mg. just before these times. The first three months of pregnancy are the most precarious, and it may be an advantage to give 5 or 10 mg. of ethisterone daily during this time. Later, the placenta will have taken over the secretion of progestin and abortion is less likely. An additional measure of some value is the administration of vitamin E (6 mg. of tocopherol daily) throughout pregnancy.

Menorrhagia.—In cases of menorrhagia in which organic causes of the hæmorrhage have been excluded, 2 mg. of progestin should be administered daily for four days immediately before the expected onset of a menstrual period. This course of treatment should be repeated during several cycles, gradually reducing the dose until only 1 mg. is given on each of the two days before a period, and then discontinuing if the hæmorrhage is then normal. Some cases which do not respond satisfactorily may benefit from the administration of a course of chorionic gonadotropin (*q.v.*), particularly cases which tend to relapse after treatment with progestin.

Metrorrhagia (*Cystic Glandular Hyperplasia*).—Doses of 5 mg. of progestin daily are usually sufficient to control

DOSAGE OF PROGESTOGENS

Condition	Progestin	Ethisterone (collaterally)	Ethisterone (alternatively to progestin)
Dysmenorrhœa	2 mg. (2 international units) three days before menstruation and 2 mg. one day before, repeated during several cycles. In resistant cases, doses of 5 mg.	5 mg. daily during three or four days before menstruation	10 mg. daily for three or four days before menstruation
Threatened abortion	5 mg. to 10 mg., repeat in a few hours and then 2 mg. daily several days after symptoms have disappeared; thereafter 2mg. weekly till the thirty-second week of pregnancy	5 mg. daily as in habitual abortion (see below)	Not indicated
Habitual abortion	2 mg. (2 international units) weekly until the thirty-second week of pregnancy. 5 mg. doses are desirable at times when menstruation would be expected in absence of pregnancy.	5 mg. daily or on alternate days	5 mg. daily until thirty-second week of pregnancy
Menorrhagia	2 mg. (2 international units) daily for four days before the expected onset of menstruation. Repeat during several cycles, gradually reducing the dose	—	10 mg. daily for four or five days before the expected onset of menstruation
Metrorrhagia (cystic glandular hyperplasia)	5 mg. (5 international units) daily until hæmorrhage is controlled	5 mg. to 10 mg. daily	30 mg. or more daily until hæmorrhage is controlled
Ovulatory hæmorrhage	2 mg. (2 international units) daily until hæmorrhage ceases	—	5 mg. to 10 mg. daily until hæmorrhage ceases
"After-pains"	2 mg. to 5 mg. (2 to 5 international units) repeated twenty-four hours later if required	—	10 mg., repeated twenty-four hours later if required

hæmorrhage. Hæmorrhage which may follow a few days after treatment has been discontinued is probably from a more or less normal secretory endometrium, and is virtually a normal menstruation. If the metropathia hæmorrhagica recurs, treatment must be designed to bring about normal luteinisation of the ruptured ovarian follicle. This will generally follow naturally if ovulation can be ensured, so that, theoretically, serum gonadotropin may be given during the immediate post-menstrual fortnight and symptomatic treatment with progestin as indicated above may be given as necessary.

Ovulatory Hæmorrhage.—Ovulation may be followed immediately by "spotting" or by hæmorrhage of clinical significance. This may be preceded by "mittelschmerz," and both pain and hæmorrhage can be controlled by giving 2 mg. of progestin daily until the condition is relieved.

"*After-pains.*"—2 to 5 mg. of progestin given a few hours after delivery and repeated in twenty-four hours if necessary is usually sufficient to give relief from "after-pains".

Sterility.—Strictly speaking, sterility is not an indication for progestin, but it is indicated in those cases of extremely early habitual abortion in which the fertilised ovum is not retained by the endometrium because the secretory or progestational phase of the endometrium is not fully developed. Doses of 2 mg. of progestin are given twice weekly during the second half of the intermenstruum, and 5 mg. of ethisterone may be given during the same period on each of the days on which progestin is not given.

Amenorrhœa.—This is a somewhat unconventional indication for progestin; it was suggested by Zondek (*Journ. Amer. Med. Assoc.* Feb., 28, 1942, p. 705).

If a normally menstruating woman receives 10 mg. of progestin daily for five days during the early part of the intermenstruum, uterine hæmorrhage will follow, sixty to seventy-two hours after the last injection. Such hæmorrhage is from a thin mucous membrane, and indicates that the formation of a proliferative endometrium is not a prerequisite for the production of uterine hæmorrhage. This observation led Zondek to administer progestin for the treatment of amenorrhœa. Quoting from the summary of his paper, Zondek suggests :—

1. For secondary amenorrhœa of more than two years' duration, a total of 50 mg. of progesterone distributed over from two to five days.

2. For secondary amenorrhœa of less than two years' duration, the same dosage, or a total of 25 mg. of progesterone with from 2·5 to 5 mg. of œstradiol benzoate distributed over two days.

3. For primary amenorrhœa and castration amenorrhœa, a total of 50 mg. of progesterone with from 2·5 to 5 mg. of œstradiol benzoate distributed over five days.

It will be noted that progestin alone is effective, but half the dose is effective if œstradiol benzoate is given. The œstradiol can be given at the same time as the progestin; indeed, the solutions of the two hormones may be mixed in the syringe and injected at the same time.

It is this work which appears to have given the first indication of a synergistic effect between œstrogens and progestogens. A more recent development of this finding is commented upon in the previous chapter (p. 99).

Progestogen Overdosage

Adverse effects from giving progestogens in excessively large doses do not appear to have been reported. Administration over unduly long periods may inhibit menstruation and disturb the menstrual cycle. Similarly, administration at inappropriate times may also result in irregular hæmorrhage.

Supplementary Note on Relaxin

In attempting to prepare progestationally active extracts of the corpus luteum of sows, Hisaw, in 1927, prepared an acid-alcoholic extract which, in addition to a progestational effect, produced relaxation of the pelvis of guinea-pigs. Lipoid extracts fail to produce this effect, so that it is clear that some substance insoluble in oil is responsible. Three years after his original observation, Fevold described a unit, the minimum amount of hormone which causes a definite loosening of the pelvic ligaments of a guinea-pig within ten to twelve hours after a single injection.

Hisaw *et al.* published their observations on relaxin in 1930 (*Journ. Amer. Chem. Soc.*, **52**, 3340), and nothing more on

the chemistry of this substance until three papers by Hisaw and his collaborators appeared in *Endocrinology*, February, 1944.

Relaxin has been found in the blood of several species of pregnant animals, the placentæ of rabbits and the corpora lutea of sows. It seems likely that it is formed in the corpus luteum and placenta, as is progestin.

Chemically relaxin appears to be a peptide. It has a nitrogen content of 11 per cent. and is destroyed by pepsin, trypsin, alkalis and by oxidising agents. It is slightly soluble in water and in 95 per cent. alcohol, but insoluble in the common organic solvents. Acid solutions are stable for at least a year.

A more precise unit is defined in this paper than was previously suggested by Fevold. It is " that amount of hormone which induces, six hours following injection, an unmistakable relaxation of the symphysis pubis in about two-thirds of a group of twelve castrated female guinea pigs weighing between 350 and 800 grammes and pretreated with 0·83 mg. of œstradiol daily for four days ".

The yield of relaxin was 120 mg. from 120 grammes of fresh sow corpora lutea.

The blood of rabbits contains 0·2 guinea-pig units per c.c. during the first nine or ten days of pregnancy. There is a rise during the fifteenth to twenty-first days (during the period of greatest development of the placenta). Thereafter it remains at a level of 10 units per c.c. until parturition, and three days after, it has practically disappeared. It is excreted in the urine.

It seems likely that relaxin is formed under the influence of œstrogenic hormone and of progestin. Large doses of œstradiol or of progestin (after pretreatment with small doses of œstradiol) produce a similar effect, but only after a longer time than after injection of relaxin itself. Relaxin produces relaxation lasting six to eighteen hours, whereas with progestin it may last for twenty-four to one hundred and twenty hours. Further, one guinea-pig unit produces a degree of relaxation comparable with that produced by 10 mg. of progestin. Œstrogens appear to increase the effect of relaxin by sensitising the pelvic ligaments as well as by activating the secretion of further quantities of relaxin, chiefly by the placenta and fœtal membranes.

Relaxin has been shown to be present in the serum and urine of pregnant women.

CHAPTER X

TESTICULAR HORMONES (ANDROGENS)

THE effects of orchidectomy have been well known from ancient times, and it is probably a matter for speculation as to when it was first thought that such effects were due to deprivation of an internal secretion. Serious attempts to isolate the testicular hormone were started by Brown-Séquard, who reported marked effects upon himself of aqueous extracts of dog and guinea-pig testes. His results were not substantiated, and this is not surprising in view of the fact that the testicular hormone is fat-soluble and not water-soluble.

That seasonal and developmental changes in male animals are brought about by an internal secretion of the testes was demonstrated by Nussbaum, who showed that implantation of testicular tissue into the dorsal sac of castrated frogs resulted in the development of the thickened pad of skin on the first digit of each forearm and of the muscles of each forearm characteristic of the male in the mating season.

Estimation of the potency of extracts, and therefore the ultimate isolation of the hormone, became possible as a result of Pézard's demonstration of the fact that increase in the size of the comb and wattles of capons follows the injection of active preparations.

McGee *et al.* in 1927 described the preparation of an active extract of testes, and an improved method was reported by Gallagher and Koch two years later.

Testicular hormone was first isolated in pure form by Butenandt, and its structure was determined by him. The substance he isolated is now known as androsterone (3-*cis*-hydroxy-17-keto-androstane).

Testosterone, the principal testicular hormone, was isolated in 1935 by David, Dingemanse, Freud and Laqueur. Butenandt and Ruzicka synthesised it independently in the same year.

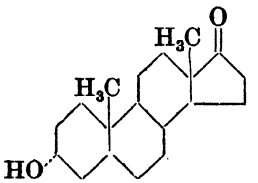
Testosterone is not inactive when administered orally, but large and expensive doses must be given by mouth to produce

satisfactory results. This difficulty was largely overcome in 1938 by the introduction of methyltestosterone.

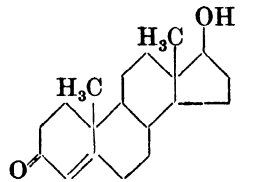
Unesterified testosterone is absorbed through the intact skin, and is used, therefore, in ointments. Suppositories have recently (1945) been introduced for rectal administration.

Chemistry of the Androgens

The androgenic hormones are derivatives of androstane which may be regarded as 10-methylœstrane (see œstrane, p. 77). As has already been stated, the first active androgen to be isolated and identified was androsterone, 3-*cis*-hydroxy-17-keto-androstane. It will be noted that this substance has a fully saturated sterol nucleus, and, in accordance with what appears to be a general rule, it is less active androgenically than is the principal testicular hormone, testosterone (3-keto-17-hydroxy-Δ^4-androstene), which has an unsaturated linkage between carbon atoms 4 and 5.

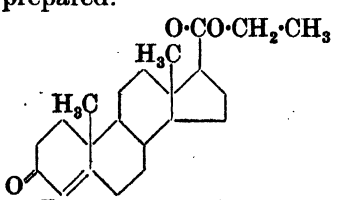


Androsterone

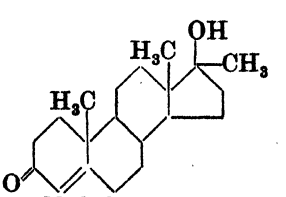


Testosterone

Like the other sterol hormones, the androgens are reasonably stable under all ordinary conditions, but their hormonal properties are enhanced and prolonged by esterification. Thus testosterone propionate is employed almost exclusively in solutions for parenteral administration. For oral administration methyltestosterone has been found to be effective and is in general use. Synthetic non-sterol androgens analogous to the synthetic œstrogens (stilbœstrol, dienœstrol etc.) have not been prepared.



Testosterone propionate



Methyltestosterone

Testosterone itself is used to a limited extent in ointments when topical application is required.

Reduction of androsterone yields androstanediol (3 : 17-dihydroxyandrostane), and this is stated to have an androgenic activity comparable with that of testosterone. Androstanediol has been used to some extent clinically, but it has now been almost entirely replaced by testosterone propionate.

A further substance, dehydroandrosterone, with slight androgenic activity has been isolated from male urine, and it has been suggested that it is an intermediate in the natural synthesis of testosterone, of which it is an isomer. Dehydroandrosterone is Δ^5-androstene-3-*trans*-ol-17-one.

Androgenic substances are secreted by the suprarenal cortex, and in the female masculinisation may result from excessive secretion from cortical tumours.

Methods of Administration of Androgens

In the male the most generally satisfactory method of administration of the androgens is by the intramuscular route, employing an oily solution of testosterone propionate. This method is also employed in the female in cases of otherwise intractable uterine hæmorrhage, but in the other relatively rare instances in which the androgens are indicated in the female some other method is preferable in order to avoid the risk of masculinisation.

As has already been mentioned, free testosterone may be administered percutaneously in the form of an ointment. The indications for this are few, but testosterone ointment may be of value in some cases of hypogenitalism or of impotence in the male collaterally with parenteral administration of the propionate. In the female, the ointment may be used on the breasts in cases of mastitis or on the clitoris as a means of producing development and thus of reducing frigidity. The use of testosterone locally in this way involves appreciably less risk of causing masculinisation.

Rectal suppositories of testosterone provide a means of giving the androgen so that the effect is exerted predominantly on the pelvic organs, and, in addition, it is suggested that absorption is more satisfactory from the rectal mucosa than is the sublingual absorption of methyltestosterone. Because

androgen absorbed from the rectum is active mainly in the pelvic region, the suggested uses for testosterone rectally are, in the female, irregular functional uterine hæmorrhage and some cases of dysmenorrhœa; in the male, delayed puberty, nocturnal enuresis, prepuberal megacolon and "male climacteric" in which there is scrotal atrophy with irritation.

Methyltestosterone is indicated in all cases in which oral administration is desirable, as a means of augmenting parenteral therapy and in those cases in which the patient objects to parenteral treatment. Methyltestosterone is administered orally, or the tablets may be placed under the tongue. Absorption is particularly satisfactory by the latter method.

Units and Standardisation of Androgens

The international unit for androgens is the activity of 0·1 mg. of androsterone. The usual test animal is the capon, and the comparison between the known and unknown androgens is based on the effect in producing comb growth. Alternatively, immature male rats can be used, increase in weight of prostate and seminal vesicles forming the basis of comparison in this instance.

Androsterone does not appear to be an entirely satisfactory standard for the estimation of androgen potency, for it has qualitative as well as quantitative differences from other androgens and it is not comparable with esterified hormones.

However, all the androgenic substances of clinical significance can be produced in chemically pure form, so that standardisation in terms of international biological units is unnecessary. In consequence, androgens are rarely described or prescribed in doses in terms of international units. Doses in terms of weight of chemically pure substance are entirely satisfactory.

Physiology and Mode of Action of Androgens

The principal hormone of the testes, and the one to which its endocrine action is almost entirely due, is testosterone. Androsterone is an "inactivation" product of testosterone formed in the body in the course of the normal metabolism of testosterone in a manner analogous to the formation of œstrone, a stage in the inactivation of œstradiol.

Testosterone is responsible in the male for the development

of the secondary sexual characteristics at puberty. It controls the *distribution* of hair, the *growth* of which is controlled by the suprarenal cortical hormones in both male and female. The general contours of the body and distribution of body-fat are probably under the control of testosterone in the male, as they are under the control of œstradiol in the female. Development of the external and internal genitalia is controlled by testosterone, and it is of interest to note that spermatogenic tissue is maintained by it in hypophysectomised animals. In intact animals, however, exogenous testosterone in sufficient doses depresses the endocrine function of the testes indirectly via the anterior pituitary. Its action on spermatogenesis is not yet fully understood, but there is some evidence that it has a direct inhibitory effect in this process. Kenneth Walker is of the opinion that androgenic hormone is an essential factor for a stage in the maturation of spermatozoa during the time they are passing through the epididymus.

It is probable that much remains to be learned of the precise functions of the androgens, for castration by no means entirely stops the formation of androgenic hormones. The cortex of the suprarenal glands also produce considerable quantities even in normal females as well as males. The excretion of large amounts of *trans*-dehydroandrosterone in the urine is commonly a symptom of cortical tumour. Whether or not the normal cortical gland substance or tumour substance actually produces androgenic substances is not finally established. They have been found in cortical extracts, but there is a possibility that they appear as artefacts during the process of extraction. On the other hand, androgens are present in the urine of castrates, but these may be breakdown products of normal cortical hormones.

The precise indications for the use of testosterone are those conditions in which it is definitely known that the underlying cause is testicular hormone deficiency, eunuchoidism, hypogonadism and impotence. Premature senility may be relieved in part with testosterone, but it must always be borne in mind that androgens are not complete " rejuvenators ". Potency may be temporarily restored and some of the more distressing and obvious symptoms of the debated male " climacteric " relieved.

It has been suggested that benign prostatic hypertrophy is the result of declining secretion of testicular hormone, with

consequent preponderance of œstrogenic hormone. Assuming that this hypothesis is correct, it is reasonable to expect that the administration of androgens would arrest the progress of the hypertrophic process, if not actually reverse it. The hypothesis has not been confirmed, however, and the results of its clinical application are equivocal.

Testosterone has been credited with an action on the peripheral blood-vessels, and in consequence has been recommended for the treatment of angina pectoris. Here again clinical results have been variable, and the treatment appears to be falling into disuse.

Nothing is known of the precise mode of action of androgenic hormones, and it is probable that the precise limits of their therapeutic possibilities will not be known until this information is available. It is well established that although the steroid sex hormones cannot be separated categorically into male and female groups, the typical androgens and œstrogens are to some extent physiological antagonists. Thus the androgens are sometimes used for the treatment of functional disorders in the female brought about by excessive œstrogen secretion.

In this connection it is of interest to recall that in some respects progestin is chemically intermediate between œstrogens and androgens. Progestin is regarded as being a physiological antagonist of the œstrogens, but not so actively as the androgens. It is reasonable, therefore, to use progestin for the treatment in " hyperœstrogenic " states in most cases and to reserve androgens for the more intractable cases. This procedure reduces the danger of causing transient but somewhat embarrassing virilisation or masculinisation in women. When it is essential to use an androgen in treating gynæcological disorders it is an advantage to use methyltestosterone rather than testosterone or its propionate, as it has been shown that the methyl compound is somewhat less masculinising than the testicular hormone itself.

Testosterone propionate has been given in cryptorchidism, but it seems that its effect on testicular size is negligible, and that it has no perceptible effect in bringing about migration of the testes into the scrotum. On the other hand, it has a marked effect on all other secondary sexual characteristics. Thus androgen therapy is contraindicated in cryptorchidic

cases when treatment is begun before the patient is fourteen years of age. Even after this age, androgens should not be given to cryptorchidic patients unless it is desirable to correct genital infantilism as quickly as possible. This is not likely in the majority of cases, for although retention of the testes in the abdomen inhibits spermatogenesis, it does not inhibit the secretion of androgenic hormone.

INDICATIONS AND DOSAGE OF ANDROGENS

Condition	Dosage
Specific Indications in the Male	
Eunuchism (complete)	Testosterone propionate 25 mg. twice weekly, or methyltestosterone 15 mg. to 20 mg. daily
Eunuchoidism and Hypogonadism	Testosterone propionate 5 mg. to 10 mg. twice weekly, or methyltestosterone 10 mg. to 15 mg. daily
Impotence (as distinct from sterility due to dyspermatogenesis)	Testosterone propionate 10 mg. twice weekly, or methyltestosterone 15 mg. daily
Male "climacteric" and Premature senility	Testosterone propionate 5 mg. to 10 mg. twice weekly, or methyltestosterone 15 mg. to 20 mg. daily
Specific and Otherwise Intractable Indications in the Female	
Metrorrhagia	Testosterone propionate 25 mg. on alternate days for three doses, or methyltestosterone 25 mg. to 35 mg. daily for three or four days
Mastodynia and Chronic mastitis	Testosterone propionate 10 mg. twice weekly, or methyltestosterone 15 mg. to 25 mg. daily.
Equivocal Indications	
Angina pectoris	Testosterone propionate 25 mg. once to three times weekly
Benign prostatic hypertrophy	Testosterone propionate 10 mg. twice weekly for six weeks, then 5 mg. twice weekly
Cryptorchidism (in certain cases only and collaterally with chorionic gonadotropin when treatment is begun well after the normal age of puberty—see text, above)	Testosterone propionate 10 mg. twice weekly, or methyltestosterone 10 mg. to 15 mg. daily

The doses in this table are those for intramuscular injection in the case of testosterone propionate and for oral or sublingual administration in the case of methyltestosterone. References to other methods of administration of androgens have been made on page 127.

Testosterone ointment is prepared containing 2 mg. or 25 mg. per gramme.

Overdosage of Androgens

Generally speaking, untoward effects of excessive doses of androgens are not permanent, and the patient will return to normal if treatment is discontinued. The effects produced are, in the male genital hyperplasia and, to some extent, priapism. In the female masculinisation is likely to result. This will be preceded by amenorrhœa and be manifested eventually by mammary atrophy, male distribution of facial and body hair, deepening of the voice and enlargement of the clitoris.

In young males sexual precociousness will be produced. This is reversible, but the precociousness may be accompanied by closure of the epiphyses, with consequent stunting of growth and disproportionate enlargement of the extremities. These latter effects are not reversible, and androgens should only be given to adolescents and young boys in relatively small doses and in exceptional circumstances.

CHAPTER XI

SUPRARENAL CORTEX HORMONES (CORTICOSTERONES)

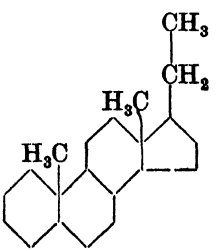
THOMAS ADDISON, in 1855, described a condition characterised by " anæmia, general languor and debility, remarkable feebleness of the heart's action, irritability of the stomach, and a peculiar change of colour of the skin." Similar symptoms are produced by adrenalectomy as well as by traumatic shock.

Degeneration of the adrenal (suprarenal) cortex was observed, and this led to attempts to prepare active extracts. Some success was achieved by Stewart and Rogoff (1927), but Swingle and Pfiffner's extract (1931) was much more successful. This last extract is still in common use, and appears to have advantages over the pure individual hormones subsequently isolated and synthesised.

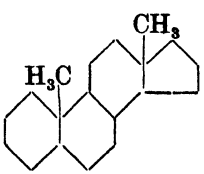
Chemistry of Suprarenal Cortex Hormones

The cortex of the suprarenal glands produces a considerable number of steroid hormones to which seven or eight functions have been attributed. The important substances in this group are contained in the extract described by Swingle and Pfiffner, known by the non-proprietary name, " cortin."

allo-Pregnane is the parent substance of most of the cortical hormones, although a few are derivatives of androstane. *allo*-Pregnane might be regarded as a 17-ethyl derivative of androstane. The structures of these compounds are as follows :—

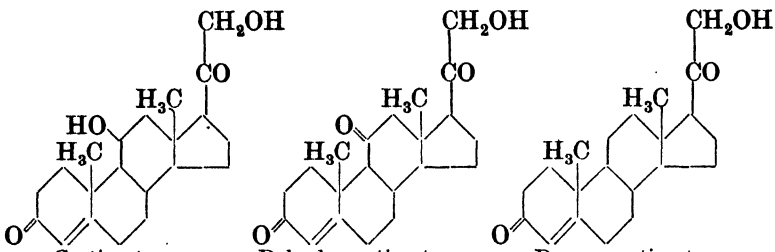


allo-Pregnane



Androstane

A characteristic of the principal cortical hormones is the presence of a keto- or a hydroxy-group attached to carbon atom 11. Examples of this are corticosterone and dehydrocorticosterone, the two substances which, singly, probably most nearly represent the qualitative action of the whole gland. The structure of these substances, together with that of desoxycorticosterone (deoxycorticosterone), the most widely used single hormone, is given below.



Desoxycorticosterone (now officially known as desoxycortone) is the only one of the individual hormones used alone to any appreciable extent, and this usually in the form of its acetate.

Administration of Suprarenal Cortex Preparations

Extracts of suprarenal cortex and solutions of desoxycortone acetate are administered by intramuscular injection. Experimentally, pellets of the latter substance have been implanted subcutaneously. This appears to be a reasonable procedure, for the ideal is to provide for continuous administration simulating the natural secretion of hormone.

When the patient is in a critical condition, glucose saline solution by the intravenous route is necessary, and the suprarenal cortex extract may be added to this.

Units and Potency of Suprarenal Cortex Preparations

There is no international unit for suprarenal cortex hormones, although biological units (dog units and rat units) have been suggested. These are unreliable, however, and the potency of extracts is best indicated by a statement as to the method by which the extract was made (usually that of Swingle and Pfiffner), together with a statement of the weight of gland

substance from which each c.c. is derived. The extracts now generally used represent 75 grammes per c.c.

Desoxycortone and its acetate are prepared synthetically in a chemically pure state. Units are therefore unnecessary, and doses are indicated in terms of weight of pure substance.

Mode of Action of the Suprarenal Cortex Hormones

The individual hormones of the suprarenal cortex differ qualitatively as well as quantitatively in their action, but it is not yet possible to state the precise function of each of the known hormones nor to indicate to what extent they overlap in their actions. Further, there appears to be some unexplained relationship between the functions of the cortex and the medulla of the gland as indicated by the pigmentation of the skin in Addison's disease and by the high concentration of ascorbic acid in the cortex and its function in the medulla.

Nothing is known of the chemistry of the action of suprarenal cortical hormones, although they evidently play many important rôles in diverse chemical processes of metabolism.

Physiological Actions of Suprarenal Cortical Hormones

Levy Simpson has stated that 70 per cent. of cases of suprarenal cortical deficiency are the result of tuberculosis of the suprarenals. Uncomplicated cases of Addison's disease are therefore relatively uncommon, although various degrees of deficiency short of the complete syndrome are not infrequent. Further, one or more abnormalities characteristic of suprarenal deficiency are frequently produced by trauma, shock or infection. It is still uncertain which of the symptoms are primary and which are secondary to the suprarenal deficiency, but they include or are attributable to the following :—

1. Muscular fatigue
2. Pigmentation
3. Low blood pressure
4. Gastro-intestinal derangement
5. Disturbance of salt and water metabolism
6. Disturbance of amino acid metabolism

Muscular exhaustion may be extreme, and it is often accompanied by apathy. This may well be a secondary

condition due simply to the low blood-sugar level. It has been suggested that the oxygen atom on C_{11} gives the carbohydrate controlling properties, and these hormones may be concerned in the conversion of protein to carbohydrate.

As has already been mentioned, the relationship of the suprarenal glands to pigmentation is a mystery. Even cortin—whole suprarenal gland extract—does not completely eliminate the pigmentation of Addison's disease. Ascorbic acid given collaterally may effect some further decrease, but some residual pigmentation remains.

Low blood pressure is probably the result of a combination of various factors, such as diminished blood volume and myocardial insufficiency. Similarly, disturbances of gastro-intestinal function are probably symptomatic of several factors, notably dehydration and the disturbed carbohydrate metabolism. Thus at least two suprarenal hormones seem to be involved: desoxycortone, which prevents loss of water and sodium and corticosterone (and dehydrocorticosterone), which is the carbohydrate-controlling hormone.

Finally, disturbances of amino-acid metabolism are probably associated with the carbohydrate disturbance (failure of formation of carbohydrate from protein) and the deranged pigment metabolism (for the pigment has amino-acid precursors, tyrosine and dihydroxyphenylalanine—" dopa ").

Clinical Uses of the Suprarenal Cortex Hormones

Primarily " cortin " and desoxycortone acetate are used in the treatment of Addison's disease. Complete relief is not generally attainable, but something very near it can be produced if dosage is adequate and is augmented with sodium chloride (10 to 20 grammes daily may be necessary) and ascorbic acid.

Cortin rapidly relieves the extreme weakness, exhaustion and apathy of Addison's disease. The appetite is improved and some of the lost weight is regained. The vomiting, which is so marked in the crises of the disease, is also rapidly brought under control. The high potassium and low sodium levels of the blood are also corrected, especially if sodium chloride is given collaterally. Lowering of the high blood urea of Addison's disease is so constant an effect of cortin administration that this has been employed as the basis of a

method of estimating the potency of extracts of the suprarenal cortex. Cases of Addison's disease in relapse often have a low blood volume. Cortin will not correct this, but if adequate fluids are given together with the cortin the blood volume returns to normal. In harmony with the relief of weakness brought about by cortin is the elimination of creatinuria, a symptom of muscle disorder.

Pigmentation is only partially overcome by cortin and by vitamin C. The hypotension may be eliminated, but in many cases there is no appreciable improvement.

Cortin has been used with some degree of success in a few other conditions having some symptoms in common with Addison's disease. These include progressive muscular atrophy, muscular dystrophy, enteric fever, erysipelas, undulant fever, hyperemesis gravidarum, infantile marasmus and post-operative dehydration.

The use of suprarenal cortex extract is decreasing in favour of synthetic desoxycortone acetate ("DOCA"), and this substance may be employed in many instances when suprarenal cortex extract is indicated. This hormonal substance appears to possess the principal actions of the whole gland extract, but certain precautions are necessary when it is used. Hypoglycæmia, hypertension and œdema have been produced in some patients, and a watch should be kept for the appearance of any of these. The use of the synthetic substance, unlike that of the cortical extract, should not be combined with the administration of extra sodium and water, nor need it be accompanied by a decreased potassium intake. If these precautions are taken it is probable that there will be no marked incidence of complications or reactions to the treatment.

Dosage and Administration of Suprarenal Cortex Extract and Desoxycortone Acetate

It appears that small doses of suprarenal extract given at relatively frequent intervals exert a more marked effect than a larger amount given in a single dose or a smaller number of doses. The whole gland extract may be given intravenously, preferably diluted with normal saline, but the intramuscular route is more commonly employed. This route is also

suggested for desoxycortone acetate, which is issued in solution in oil. Small doses of the extract or of desoxycortone acetate may be given by deep subcutaneous injection.

When not in use, it is advisable to keep the extract on ice in order to inhibit loss of activity.

For maintenance of patients with Addison's disease tablets of desoxycortone acetate have been implanted under the skin. Administration in this way is, of course, not suitable for patients in crisis.

Condition	Dose	Remarks
Addison's disease	In crisis or severe deficiency, 15 c.c. to 40 c.c. of extract daily (intramuscularly or, diluted with saline, intravenously) In mild or moderate deficiency, 3 c.c. to 10 c.c. of extract daily Alternatively 5 mg. to 10 mg. of desoxycortone acetate daily at the beginning of treatment, reducing to 5 mg. to 15 mg. weekly as a maintenance dose, intramuscularly	Desoxycortone acetate should not be used alone in crisis or if the principal symptoms are not controlled. Some mild cases may be controlled with smaller doses or even with sodium chloride only. For maintenance, when a patient's reactions to the drug are known, 50 mg. to 150 mg. in tablets may be implanted under the skin.
Asthenia (hypoadrenal) (post-infective)	3 c.c. to 5 c.c. of cortin daily, or up to 10 mg. of desoxycortone acetate daily, intramuscularly 2 c.c. or 3 c.c. daily or 5 c.c. on alternate days or up to 5 mg. of desoxycortone acetate daily	Similar doses may be given in pituitary cachexia.
Hyperemesis gravidarum	1 c.c. of cortin, repeated in 1 to 1½ hours and then 1 c.c. daily	
Marasmus (infantile)	0·5 c.c. daily	
Toxæmia, especially with dehydration	5 c.c. to 20 c.c. of cortin daily, in urgent cases by intravenous injection	Divided doses may be more effective than single daily doses.
Toxæmia, from severe burns	Children, 1 c.c. every two hours, adults 2 c.c. hourly of cortin	—

POSTULATED HORMONES OF UNKNOWN CONSTITUTION OR DOUBTFUL EXISTENCE

CHAPTER XII

PINEAL HORMONE

THERE is some doubt as to whether the so-called pineal "gland" is an endocrine organ at all. Anatomically it is a vestigial eye, functional in certain of the lower animals, such as lampreys, which have no true paired eyes as have the reptiles and higher Chordata. The pineal eye is probably only capable of differentiating light from dark and is not endued with true vision.

In the higher animals the pineal has a definite glandular structure and appears to be concerned in some way with metabolism. It has been stated that the pineal gland undergoes involution, the process beginning at about seven years of age and complete at puberty. On the other hand, it has been stated that there is no definite atrophy of the pineal gland at any age.

Again, statements as to the function of the pineal gland appear to be somewhat contradictory. Stewart (*Oxford Med.*, vol. 6, part 1, p. 39) states that "the pineal secretion has a profound influence upon growth and upon certain trophic functions. It inhibits development of the genital organs. Increased secretion or super-pinealism causes excessive adiposity and retards the onset of puberty. Diminished secretion or sub-pinealism, which is met with in certain teratomata of the gland occurring during childhood, causes precocious and abnormal development of the genital organs and also of secondary sexual characteristics, with mental precocity, adult type of voice etc. and one type of macrogenitosomia."

These symptoms of "subpinealism" in particular are questioned. It has been suggested that the first effect in tumour formation in the pineal gland may be hypersecretion,

as in the anterior pituitary gland (Werner, *Endocrinology*, 1942, p. 782). This suggestion is considered by Werner to be confirmed to some extent by the finding of an œstrogenic substance in the pineal gland.

Werner also suggests (*ibid.*, p. 783) that pineal œstrogen may serve to sustain pre-pubertial secondary sexual characteristics, at least in the female. It is perhaps not unreasonable to speculate still further that this necessarily small amount of œstrogen may serve to potentiate the androgen necessary for the analogous maintenance of secondary sexual characteristics in the male before puberty.

In an Annotation in the *British Medical Journal* (Feb. 24, 1945, p. 268) it is stated summarily that " Pineal tumours . . . produce true precocious puberty only in boys, and it is now accepted that they do so not by virtue of any endocrine overactivity but by pressure on the hypothalamus." No authority for this statement is given, and it is somewhat surprising in view of the marked effects on growth and sexual development produced in various animals by the administration of pineal extracts. On the other hand, pinealectomy has produced no conclusive results. However, this may well be if the main, or one of the main pineal hormones is an œstrogen. It might be expected that some indication of the function of the pineal gland would be given from a consideration of the symptoms accompanying pineal tumour. Such tumours are rare, however, and such symptoms as have been reported may be the result of pressure on the brain, hypothalamus or pituitary gland.

The question of whether the pineal is an endocrine organ or not is therefore not finally decided. Suggestions have been made as to its function, but proof or rejection of these is difficult, and present knowledge is probably insufficient as a basis upon which a decision could be formulated. The clinical use of pineal preparations is therefore not justifiable, and administration should be undertaken only for experimental purposes in elucidating the function of the gland or with a view to isolating an active principle.

CHAPTER XIII

THYMUS HORMONE

THE nature and function of the thymus gland have remained more or less a mystery from ancient down to modern times. The organ is present in primitive fishes and in all other animals above them in the evolutionary scale. There seems to be some reason for attributing to it endocrine functions, but in origin it is an epithelial structure, and at birth or some later period in life it appears to undergo lymphoid transformation. The thymus and the parathyroids arise from the third, or sometimes the fourth branchial clefts. It has been suggested, therefore, that the thymus may share some of the functions of the parathyroids or have some similar or related function.

Agreement has not been reached as to the normal size of the thymus at various ages. It probably reaches its maximum size during the period of greatest growth, undergoes involutionary changes at puberty and then diminishes in size. The much-discussed status thymicolymphaticus is said to be associated with abnormal enlargement of the thymus gland, but whether the hyperplastic gland is responsible for the symptoms by reason of some abnormal secretion or mechanically by reason of its enlargement is not clear. It has also been suggested that an abnormal thymus secretion may be the causative factor in myasthenia gravis.

In this last condition, the secretion postulated is likened to curare, the effect of which is to inhibit muscular activity, seemingly by interfering with the acetylcholine mechanism of muscular control. The probability of this suggestion seems to be heightened by the increasing success which seems to follow thymectomy undertaken for the relief of myasthenia gravis. It does not necessarily follow, however, that the myasthenia-causing factor of the thymus is a hormone or even a hormonal decomposition product. It may be that this as yet hypothetical factor is normally an essential component of the acetylcholine inactivating mechanism, associated perhaps with cholinesterase

and acting similarly to it at some other point in the choline–acetylcholine cycle. Hypersecretion of such a factor is conceivable as a cause of myasthenia gravis, but this factor could not be considered to be a hormone, but rather an enzyme, or perhaps a "neurohormone," comparable with, but antagonistic to, acetylcholine.

If the thymus has an endocrine function it is more likely to be associated with the suggested function of the thymus in controlling growth and inhibiting the appearance of the secondary sex characteristics and perhaps the onset of puberty. In this connection it may be noted that thymus deficiency (or thymectomy) is said to result in the laying of shell-less eggs by pullets and pigeons. This, together with the suggestion that enlargement may cause rickets or osteomalacia in humans is significant from the postulated similarity of its function to that of the parathyroids. A further point tending to confirm this similarity is the suggestion that enlarged thymus is found in patients with acromegaly.

Some curious effects on growth have been reported from the administration of a thymus extract to rats during the course of several generations. Briefly this results in marked precocity of the offspring of treated parents after the second generation. The young show increased growth and development, small adrenal glands and lymphatic hyperplasia. They do not exhibit symptoms comparable with those postulated as those of status thymicolymphaticus.

Thus it will be obvious that the present state of knowledge of the thymus gland and its function is such that no recommendations for its clinical use can be made. Preparations of thymus gland have been put on the market, but their use is probably not justifiable. In particular, thymus extract in combination with posterior pituitary extract has been suggested as an improvement on simple posterior pituitary extract, but such combinations have been rejected by the Council on Pharmacy and Chemistry of the American Medical Association as being dangerous, in that they may give rise to a false sense of security. It was suggested that the thymus extract mitigated the adverse and violent action of the pituitary extract, but it appears that there is no justification for this assumption.

CHAPTER XIV

PROSTATE HORMONE

THE prostate is certainly a gland of *external* secretion (an "exocrine" gland), but there is considerable doubt as to whether it has an endocrine function in addition.

The prostate gland surrounds the urethra at its posterior end as it emerges from the bladder. Its exocrine function is to supply an alkaline diluting fluid for the semen. This fluid is secreted by the fifteen to thirty branched tubular glands in the prostate which join the ejaculatory ducts as they pass through the gland. The prostatic excretion serves to promote the motility of the spermatozoa and perhaps to neutralise in part vaginal secretion which is somewhat too acid to permit of prolonged viability of the spermatozoa.

The evidence for an endocrine function of the prostate is remarkably meagre. It has been said that prostatectomy may be followed by "prostatic neurasthenia," and the administration of the gland has been recommended for the relief of this. Prostatic substance has also been given in testicular atrophy, but there is no evidence that the prostate has any action on the testes, although testicular hormone is undoubtedly "prostatotropic" to the glandular or epithelial tissues of the prostate. Finally, prostate gland substance has been given for the treatment of benign prostatic enlargement, but the rationale of this treatment is not clear and its efficacy is open to doubt.

Whether the prostate has an endocrine function or not is thus debatable, and the most that can be said at the present time is that the point is still *sub judice*.

Benign Prostatic Enlargement

The chief endocrinological interest in the prostate gland is centred in the endocrine treatment of prostatic enlargement. The most common type of this is benign hypertrophy or adenoma of the prostate. In this condition there is an increase in the amount of glandular tissue, the prostate becoming large and soft. The increase in bulk of the gland

causes a constriction of the urethra, in consequence of which there is increasing difficulty in micturition proceeding eventually to urinary retention. The ætiology of this condition is not clear, but it has been suggested that it is a consequence of an undue preponderance of œstrogenic (or rather gynœcogenic) hormone resulting from a decrease in the secretion of androgenic hormone by the testes. This theory of the causation of benign prostatic enlargement has met with some measure of acceptance, but acceptance is not universal. On the basis of the theory, the enlargement should be prevented or arrested if androgenic hormone is administered so as to eliminate the œstrogen preponderance. Some measure of success has been claimed, and it has even been suggested that some regression in the size of the gland has been produced. The treatment has not been an unqualified success, however, and for this reason the validity of the theory has been questioned.

In this connection there is a point which seems to merit investigation. It has been suggested that in addition to the androgenic hormone, testosterone (secreted in the interstitial tissues of the testis), there may be another testicular hormone, secreted by the germinal epithelium (*Brit. Med. Journ.*, Dec. 9, 1944, p. 759). The name " inhibin " has been suggested for this hormone, and its function appears to be the control (inhibition) of the anterior pituitary and of the prostate. More recently it has been suggested that the hormone of the testicular germinal epithelium is œstradiol. If this is to be understood to imply that inhibin is œstradiol, there appear to be certain difficulties not easy to explain. For example, inhibition of the anterior pituitary by œstradiol would probably involve the secretion of such an amount of œstradiol as would nullify the effect of testosterone and have an adverse effect on the prostate.

Since testosterone is not entirely satisfactory in the treatment of prostatic enlargement, therefore, and since there is reason to question whether inhibin is really œstradiol, it may well prove profitable to reinvestigate the question of the existence and nature of inhibin. Perhaps benign prostatic hypertrophy is a manifestation of testicular atrophy or hyposecretion in general and of hyposecretion of inhibin in particular.

Another point of interest which appears to merit further

investigation in this connection is the suggested occurrence of the prostate in the female.

The prostate gland is commonly regarded as being an exclusively and typically male organ, but several references appear in the literature to an analogue of the prostate in the female. Folsom and O'Brien (*Journ. Amer. Med. Assoc.*, Feb. 20, 1943, p. 573) quote earlier references and report upon six cases of " benign prostatic hypertrophy " in women whose ages averaged 59·6 years and in one woman of 25 years. The women complained of symptoms virtually identical with those occurring in men and which were relieved by " prostatic resection."

It is possible that the " female prostate " consists solely of muscle-tissue and is simply the internal vesical sphincter. Whether this is so or whether the organ in the female does include glandular tissue in addition is not clear, but the question remains as to the nature of the endocrine mechanism which may be responsible for hypertrophy of the internal urethral sphincter in the female.

Prostatic Carcinoma

The internal sphincter of the urinary bladder consists of prostatic muscle-tissue, and the second and less common form of prostatic obstruction is a result of a carcinomatous process in this tissue. Whereas the benignly hypertrophied prostate is soft, the carcinomatous prostate is hard.

The treatment of prostatic carcinoma with synthetic œstrogens has been considered in the Supplementary Note to Chapter VIII (p. 109). The carcinomatous lesion in the prostate is in the stroma of the gland, but whether this tissue is affected directly or indirectly by œstrogens is not known. According to one theory, spread of the lesion in the stroma is inhibited by the increased activity of the epithelial tissues—that is to say, the carcinoma in the stroma is countered by a benign hypertrophy in the epithelium !

To summarise, it is of the utmost importance to differentiate between benign and malignant enlargement of the prostate before embarking upon any course of treatment, for if diagnosis is inaccurate the wrong treatment is likely to be given, and the condition is then likely to be aggravated.

PART II

VITAMINS

CHAPTER XV

DEFINITION

VITAMINS are so diverse in their chemical nature and in their functions that a precise and comprehensive definition is not easy to formulate. For many years after the existence of vitamins was first postulated and until quite recently it was considered to be something in the nature of a joke to ask, " What is a vitamin ? have you ever seen one ? " Now, not even the most confirmed sceptic questions the existence of most of the vitamins, for not only have most of the well-known vitamins been isolated and prepared in crystalline form, but many of them have been prepared synthetically and been proved to be identical with the naturally occurring substances.

Vitamins are usually classified into two main groups : a group which is soluble in oils and fats, and a group which is soluble in water. Now that the part they play in physiological processes is more clearly understood, the basis of classification might be whether they act in the form of the vitamin itself or chemically combined with some other substance. In the first group would be vitamins A and C and possibly D and K, and in the second group vitamin B_1, riboflavine and nicotinamide. Such a classification could not as yet be completed, however, for the chemistry of the action of a number of the vitamins is still unknown.

It can be stated that all the vitamins whose mode of action is known are " organic catalysts ", and this is the most concise definition which has been formulated. Less concisely, but probably more accurately, vitamins might be defined as enzymes or essential prosthetic groups of enzymes and coenzymes necessary for the maintenance of normal metabolic processes (usually of oxidation) and which are not elaborated in the

body, but which must be ingested from dietary sources. Such a definition is probably accurate as far as it goes, but it is not sufficiently comprehensive to include the provitamins nor sufficiently exclusive to exclude the " trace elements " such as copper or manganese. A vitamin is, by definition, a substance which the body cannot synthesise, but a provitamin can be converted into the corresponding vitamin in the body. There are three well-established examples of this; carotene is hydrolysed in the liver to form vitamin A, nicotinic acid is converted into nicotinamide and 7-dehydrocholesterol is converted by ultra-violet into vitamin D in the skin. Thus a vitamin or a provitamin contains an essential chemical radicle or grouping which the body is incapable of synthesising. A hormone, on the other hand, is entirely synthesised in the body and, like a vitamin, subsequently plays an essentially enzymatic rôle. This, briefly, is the fundamental difference between the two types of compounds. Another difference is that the hormones, being essentially " messengers," are by definition mobile, and readily conveyed from their gland of orgin by the bloodstream to their site of action in distant viscera or tissues. Vitamins in their active form as enzymes or coenzymes act intracellularly and in most cases only when they have combined with proteins which are cell constituents. Thus they are static once the uncombined vitamin has been conveyed to the tissues where they are required and until they are eliminated and renewed in the course of the routine replacement of worn-out tissues throughout the body.

There is one further point about vitamins which it is desirable to emphasise here—namely, that they are required in minute amounts as compared with the other nutrients, and that they do not provide any appreciable energy, as do the fats or carbohydrates, but in many instances they do serve to make available the energy or heat contained in the nutrients required in bulk, carbohydrates, fats and proteins.

Finally, the dividing line between vitamins and the " bulk " nutrients is not always definite. For example, the *essential* amino-acids (those which the body cannot synthesise) are sometimes regarded as vitamins, although as constituents of the proteins they are also included among the " bulk " nutrients.

CHAPTER XVI

VITAMIN A

BEFORE the name "vitamin" had come into general use two accessory food factors were recognised: "fat-soluble A" and "water-soluble B". The original "fat-soluble A", or vitamin A, was subsequently found to consist of two substances, which were later called vitamin A and vitamin D. The existence of the original fat-soluble vitamin, necessary for growth, was first recognised by McCollum and Davis in 1913. Various papers by Mellanby between 1918 and 1921 provided evidence which established rickets as a deficiency disease, and Mellanby was inclined to identify the antirachitic vitamin with vitamin A. In 1922, however, McCollum, Simmonds, Becker and Shipley showed that 12–20 hours' oxidation of cod-liver oil at 100° C. destroyed the antixerophthalmic action of the oil, but not its antirachitic action. This and other evidence produced during the following year finally established that the original "fat-soluble A" consisted of two distinct substances.

Xerophthalmia, the classical symptom of vitamin-A deficiency, was generally believed to be of dietary origin many years before the discovery of vitamins. Monrad in 1917 suggested that the condition was due to deficiency of an accessory food factor, and in 1925 Findlay demonstrated that the tears of vitamin-A-deficient rabbits were deficient in lysozyme, thus accounting for the extreme susceptibility of such animals to bacterial invasion.

Night-blindness was established as a symptom of vitamin-A deficiency by Holm in 1925 and confirmed by Tansley in 1931.

Perhaps the most controversial property attributed to vitamin A is its anti-infective action. Many investigators have shown that vitamin-A-deficient animals are unduly susceptible to bacterial infection, referable in each instance to epithelial degeneration. The work of Mellanby and Green (1929–1930) seems to indicate that vitamin A may play some part in increasing resistance to puerperal septicæmia.

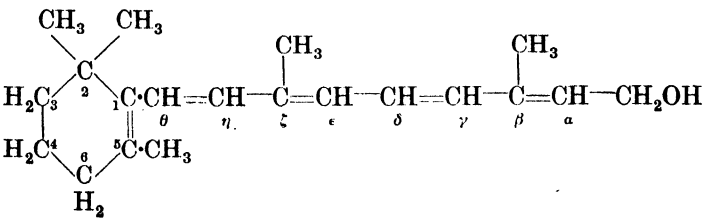
Pneumonia among native workers in a South African gold mine appears to have been favourably influenced by the administration of vitamins A and D in the form of Radiostoleum (Donaldson and Tasker, 1930). This supposed antitoxic action of vitamin A has fallen somewhat into disrepute, however, during recent years.

Several degenerative conditions of nervous tissue have been attributed to vitamin-A deficiency as well as reproductive failures in rats. The phenomena associated with reproduction, however, are probably related to vitamin-E deficiency, and the vitamin A–vitamin E relationship must be considered.

The resistance of vitamin A to alkaline saponification of the oils in which it is contained was demonstrated by McCollum and Davis in 1914, and this property of the vitamin formed the basis of many attempts to isolate it. This was not achieved, however, until 1937, when Holmes and Corbet reported the isolation of crystalline vitamin A. The pure vitamin is a pale yellow substance occurring in needle-shaped crystals which melt at 7·5° to 8° C. and do not readily solidify again.

Chemistry of Vitamin A

Vitamin A itself is an alcohol, although it generally occurs naturally in the form of esters of fatty acids. Vitamin A alcohol has the following structure :—



and the empirical formula $C_{20}H_{29}OH$.

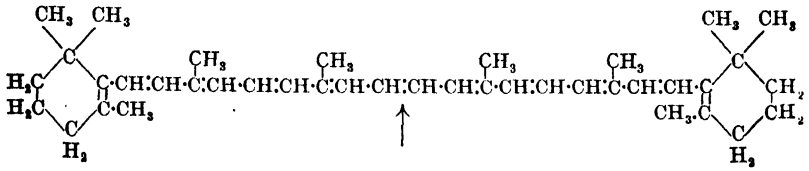
Following the accepted system of nomenclature the chemical name of this substance is :—

θ-(2 : 2 : 6-Trimethyl-Δ^6-*cyclo*hexenyl)-β-ζ-dimethyl-$\Delta^{\alpha,\gamma,\epsilon,\eta}$-octatetraene-$\alpha$-carbinol.

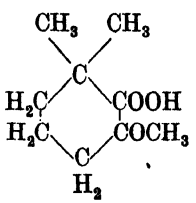
The initial letter θ (theta) of this name indicates that the ring system on the left of the structural formula is attached to the θ (eighth) carbon atom of the side-chain, counting from the

right and beginning with the carbon atom to which the alcoholic group $-CH_2OH$ is attached. The part of the name in brackets describes the ring structure, indicating the position of the three methyl groups (2 : 2 : 6-trimethyl) and the double bond (Δ^6). The position of the two methyl groups on the side-chain is given (β : ζ–beta : zeta); and the nature of the side-chain (octatetraene—an eight-carbon-atom hydrocarbon with four double bonds) is then indicated, together with the positions of the double bonds ($\Delta^{\alpha,\gamma,\epsilon,\eta}$)—that is, at carbon atoms "numbered" α (alpha), γ (gamma), ϵ (epsilon) and η (eta). Finally, α-carbinol describes the alcoholic group $-CH_2OH$ attached to carbon atom α (alpha).

Carnivorous animals obtain their vitamin A from their diet, the liver of all normal animals being rich in the vitamin. The herbivorous animals obtain their vitamin A by hydrolysis of the carotenes of plants. The most important of these is β-carotene, which has the following structure :—



Theoretically it would appear that this molecule should divide into two equal parts, and each half combine with water (one molecule to each half) to form two molecules of vitamin A. Probably, however, only one molecule of vitamin A is formed from one of β-carotene, geronic acid being formed from the second ring of the β-carotene molecule, geronic acid being :—



The other carotenes have only one ring in the molecule identical with the one characteristic of β-carotene and of vitamin A (the β-ionone ring), and these carotenes (α- and γ-) are less active than the β-form.

The vitamin A described above (axerophthol) is also known

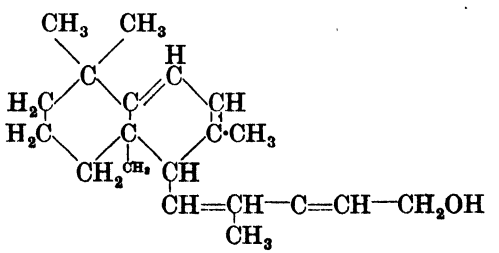
as vitamin A_1, as distinct from vitamin A_2 of fresh-water fish. Vitamin A_2 appears to be physiologically identical with vitamin A, but the molecule of the former appears to contain an extra ethylene (–CH=CH–) group between the α-carbon atom and the carbinol (alcoholic) group.

Vitamin A is stable at all ordinary temperatures, but it is readily oxidised. Its oxidation is accelerated by exposure to heat and possibly by exposure to light. Preparations containing vitamin A should therefore be kept in carefully closed, dark containers in a cool place. When dispensed, such preparations should not be mixed with oxidising agents, and unless they are to be used quickly, amber glass or other dark-coloured containers should be used.

It has been suggested that vitamin A might be protected from oxidation by the addition of hydroquinone to solutions containing it, but the amount of hydroquinone required would render such preparations unsuitable for medicinal use.

There is some evidence that indicates that vitamin E may be a natural antioxidant for vitamin A. (This will be discussed in more detail in the chapter on vitamin E.) The possibilities of applying this to the pharmaceutical problem of preserving both vitamins seems to be deserving of further investigation.

Preparations containing A are also subject to loss of potency on keeping, even when kept in the dark and in an inert atmosphere. This loss of potency is due to cyclisation of the molecule, a process which takes place in acid solution and to some extent when the isolation of the pure vitamin is attempted by molecular distillation. Cyclised vitamin A possibly has the structure indicated by the following structural formula :—



Molecular distillation is essentially distillation under very reduced pressure (about one millionth of normal atmospheric pressure), whereby the use of heat is for the most part eliminated

and heat-labile substances can be purified by distillation without decomposition. In this process, mixtures of substances can be separated, the order in which the various fractions come over being controlled, not by boiling point, but by molecular weight, the substances of lower molecular weight coming over first.

Administration of Vitamin A

Vitamin A is almost invariably administered orally in solution in oil, either the natural oil in which it occurs, refined and purified, or in vegetable oils to which the vitamin may be added after isolation. Except in diseases characterised by malabsorption of fats, vitamin A is satisfactorily absorbed when this method of administration is adopted. The prolonged taking of liquid paraffin, however, does interfere with absorption, and should therefore be avoided when vitamin A is being administered. It will generally be found that even in sprue, steatorrhœa and cœliac disease, when fat absorption is at a minimum, the oral route may still be employed if one of the high-potency solutions of vitamin A in oil is given, an adequate dose being contained in a relatively minute volume of oil. When the use of oily solutions of the vitamin by the oral route is completely precluded, vitamin A may be administered in oily solution by intramuscular injection, but this method of administration is rarely necessary. (These remarks on administration also apply to vitamin D).

Vitamin A has been suggested for use by local application as a means of promoting the healing of burns and extensive surface wounds, either as cod-liver oil or as a concentrate in vegetable oil or an ointment. The evidence of its value used in this way is somewhat inconclusive.

In the past, carotene has occasionally been administered orally in tablets or in sugar-coated pellets. Equivalent doses of carotene, however, are expensive as compared with vitamin A and supplies are not available at the time of writing.

Unit and Potency of Vitamin A

The international standard preparation in terms of which the activity of vitamin A and its preparation are expressed is pure β-carotene. Each 0·6 γ (microgram) of β-carotene

has an activity of one international unit. The precise weight of pure vitamin A to which this corresponds is not yet generally agreed upon, but it appears that vitamin A has an activity of about 3,000,000 international units per gramme—that is, three units per microgram, or about two units per 0·6 γ. The actual figure may be slightly less than this in accordance with the fact that each molecule of β-carotene on hydrolysis does not form two molecules of vitamin A. It is thus still necessary to express doses of vitamin A in terms of international units, although the pure vitamin has been prepared. (A more recent statement gives the activity of vitamin A as 4,500,000 international units per gramme.)

Estimation of Vitamin A

There is no simple chemical means of estimating vitamin A. If a colorimeter with a suitable standard colour disc is available, however, a reasonably accurate colorimetric estimation can be made by means of the Carr-Price antimony trichloride test. The greatest degree of accuracy is obtained when relatively pure " concentrates " of vitamin A are estimated. The test should therefore be carried out on the unsaponifiable fraction of the oil. Only very approximately accurate results are obtainable with natural oils which may contain variable amounts of inert substances which give the blue colour with antimony trichloride.

The test was first described by Carr and Price (*Biochem. Journ.*, 1926, **20**, 497). It is in common use as a rapid test and for confirmation of the results of the biological and spectrophotometric tests described in the first and second Addenda to the B.P. respectively.

The " blue value " of a vitamin preparation as determined by the Carr–Price test $\times 30$ gives the approximate potency in international units per gramme.

It will readily be understood that none of these three methods of estimation is suitable for use by most physicians or retail pharmacists. The Carr–Price antimony trichloride reaction might be employed, however, as a means of demonstrating the absence or possible presence of vitamin A.

Allport's " Colorimetric Analysis " (Chapman and Hall, 1945, pp. 338 and 349) should be consulted for details of

colorimetric methods of estimations of β-carotene and of vitamin A.

Physiological Action of Vitamin A

The carotenes (α, β and γ), after ingestion and absorption, are hydrolysed in the liver to form vitamin A, which is then stored in the liver in the form of esters of the higher fatty acids. Carotene, itself, appears to be of no physiological significance in animals except as a suitable source of vitamin A for the herbivora and for the omnivora, such as man. An excessive intake of carotene or severe impairment of liver function, however, can result in a condition of carotenæmia in which the carotene is widely deposited in the body, giving the appearance of jaundice. The condition is rare, has no permanent effects, and disappears when the intake of carotene is reduced.

The rôle of carotene in plants appears, superficially, to have little in common with that of vitamin A in animals. The maximum absorption of light by the carotenes is in the neighbourhood of 450 to 520 mμ, the region most active for the synthesis of carbohydrates. It seems likely, therefore, that carotenes, together with chlorophyll and other leaf-pigments, collaborate in carbohydrate synthesis. Decomposition products of carotenes play a part, at least in the green alga *Chlamydomonas eugometos*, in sex determination. There is no evidence of such a function in animals, but the action is perhaps of sufficient interest to outline here. It was given in detail in the *Journal of the Chemical Society*, February 1942, p. 79.

Carotenoid Derivatives as Sex-determining Factors for Chlamydomonas

Carotene is the source of crocetin (dicarboxylic acid derivative of the hydrocarbon chain of carotene), some of which forms crocin by combining with two molecules of the disaccharide gentiobiose. The amotile gametes of *Chlamydomonas eugametos f. simplex*, in the presence of crocin (1 part in 250 billion is active) and light of any wavelength, develop cilia and become motile. The *cis* isomer of crocetin is first formed, and this is gradually converted into the *trans* isomer under the influence of ultra-violet light, a mixture of three parts of *cis* isomer to one part of *trans* isomer being formed in twenty minutes. This

mixture is the gynotermone, and makes asexual motile cells female. If the *cis*-crocetin is exposed to ultra-violet light for thirty minutes, the ratio of 1 *cis* to 3 *trans* isomers is formed, and the mixture is the androtermone, converting asexual gametes into male gametes.

In the closely related *C. eugametos f. sinoica* a similar sex-determining mechanism operates, but the gynotermone is picrocrocin (safranal gentiobiose glycoside), and the androtermone is safranal itself, safranal being derived from the ring system of the β-carotene molecule.

The mechanism of these reactions is unknown, but in accordance with the principle of the simplest explanation probably being the correct one, it may be that the termones react with the genes, or with the nuclear proteins closely associated with their metabolism. If this is so, a related mechanism, perhaps involving vitamin A, may be a sex-determining factor in animals.

The foregoing, however, is new, and by no means fully investigated work, and any deductions from it must be highly speculative.

The ready oxidisability of vitamin A suggests that it is a factor essential for the continuance of some oxidation-reduction process or processes, the mechanism of which is unknown. The function of vitamin A aldehyde (as retinene) in the rhodopsin cycle in the retina of the eye (see page 156) is of interest, however, in this connection. The principal symptom of vitamin A deficiency is degeneration of epithelial cells, particularly those of the mucosæ of the eyes, respiratory tract and urinary tract. The first of these—degeneration of the conjunctivæ—constitutes the classical vitamin-A deficiency state, xerophthalmia. The second, degeneration of the mucosa of the respiratory tract, with resulting susceptibility the respiratory infection, provides the generally accepted reason for describing vitamin A as being anti-infective.

Affected cells become keratinised and then disintegrate. Thus the way is opened for invasive bacteria to enter the body. In addition to the mucosæ already mentioned, other areas particularly likely to degenerate as a result of hypovitaminosis-A include the uterus, vagina, pancreatic duct and salivary glands. The renal tubules, and pelvis and ureters may also be affected, and in such cases the resulting cellular debris, and perhaps

bacterial infection, have been credited with forming a nucleus upon which renal or ureteral calculi may be formed. Hair-follicles and sweat-glands also become keratinised, resulting in a further classical symptom of hypovitaminosis-A, phrynodermia or toad-skin. It is important to differentiate between this condition and one sometimes resulting from hypovitaminosis-C in which there is an exudation from the hair follicles which hardens and in which the hairs are found to be coiled up like watch-springs in the solidified exudate. There is a superficial resemblance in the appearance of the two conditions.

The skin generally becomes rough and dry, and may develop a papular eruption and pigmentation, the dryness and roughness of the skin being the first dermal symptom of the hypovitaminosis.

In addition to its rôle in maintaining epithelial integrity, vitamin A is also essential for normal growth of bone and formation of dentine of teeth. It appears that vitamin E may also be essential for normal formation of the enamel of teeth, an association which has already been mentioned in the section on the chemistry of vitamin A, and which will be considered in more detail in a subsequent chapter on vitamin E.

Symptoms of Vitamin A Deficiency

The first symptom of deficiency to appear when the intake of vitamin A is subnormal is a lengthening of the time of adjustment of the eyes to dim light after exposure to relatively bright light. If the deficiency state is more severe, then adjustment may not take place at all, and night-blindness results. It is a well-established fact that vitamin A does play some part in the processes involved in vision. In outline, the process is generally thought to be that vitamin A unites with a protein to form visual purple (or rhodopsin), which, in the retinal rods, breaks down to form visual yellow (or retinene) under the influence of light. This decomposition, it is suggested, releases energy necessary to convey the nervous impulses from eye to brain, producing the sensation of sight. In the absence of adequate reserves of vitamin A, the process of regeneration is delayed and visual acuity in dim light is impaired. Morton questions this theory (*Nature*, Jan. 15, 1944, p. 69), and suggests that although vitamin A is necessary for the formation and re-

formation of rhodopsin, it does not form part of the molecule of rhodopsin. Indeed, he gives some evidence which appears to indicate that rhodopsin is a flavoprotein enzyme—that is, that the active prosthetic group in the substance is riboflavine (vitamin B_2).

In a subsequent communication Morton shows that retinene is vitamin A aldehyde. This has been prepared from vitamin A alcohol and identified with natural retinene (*Nature*, April 1, 1944, p. 405). The rhodopsin cycle is probably complex, but the essentials of it may perhaps be summarised as follows.

Rhodopsin is dehydrogenated (" oxidised ") by light to form visual yellow (1). Retinene is hydrogenated by visual yellow to form rhodopsin and vitamin-A alcohol (2). Vitamin A alcohol is dehydrogenated possibly by triphosphopyridine nucleotide (cozymase II), giving retinene once more (3).

(1) Rhodopsin $- H_2 \xrightarrow{\text{light}}$ visual yellow

(2) Visual yellow + Retinene $\longrightarrow$ Rhodopsin + Vitamin-A alcohol

(3) Vitamin-A alcohol + cozymase II $\longrightarrow$ Retinene + reduced cozymase II

If this conception of the rôle of vitamin A should prove to be correct, all the known functions of vitamin A in the body may perhaps be uniformly conceived as indicating that it is an essential factor for certain processes in the synthesis of proteins and of their maintenance in a normal state. The majority of the symptoms of hypovitaminosis-A, for example, may be considered fundamentally to be processes of degeneration of the protein structure of the walls of the cells of the mucosæ and epithelium. The malformation of bones and teeth again is fundamentally a failure in formation, or a degeneration, of the protein matrix of these structures, and it may be that degeneration of the nerve tissues attributed to vitamin-A deficiency is of a similar type. Similarly, it may be that the postulated anti-infective properties of vitamin A are an indication that it plays some part in the elaboration of " antibodies ".

Thus vitamin A is perhaps necessary for the formation and maintenance of proteins generally, and of the more highly specialised proteins in particular. Among the latter would appear to be visual purple (rhodopsin), antitoxins (" anti-

bodies ") and the specialised proteins of the mucosæ and the nerves, as well as the organic matrix of bones and teeth. In this connection it is interesting to note that cattle deficient in vitamin A may lose the power of synthesising vitamin C (*Veterinary Record*, Aug. 5, 1944, p. 287) (*cf.* the relationship of vitamin C to protein metabolism and antibody formation; some interesting inter-relationships between the two vitamins are indicated).

Mode of Action of Vitamin A

The chemistry of the action of vitamin A is unknown, but the vitamin is almost certainly an oxidation-reduction catalyst, particularly associated with epithelial tissues and apparently concerned with structure rather than function.

From the preceding discussion it will be seen that it seems possible that vitamin A plays some essential rôle in the synthesis and maintenance of a variety of specialised and essential proteins. Confirmation of this, and the description of details of its mode of action can be produced only as a result of further investigation.

Clinical Uses of Vitamin A

The principal action of vitamin A is generally considered to be the maintenance of the integrity and healthy condition of the cells of the mucous membranes and epithelia. If this conception is extended, as indicated on p. 157, to include the maintenance of the more highly specialised protein structures and tissues generally, the indications for vitamin A fall into a logical and connected scheme which is comparatively easily remembered and in terms of which the validity of subsequently suggested uses for the vitamin may be approximately assessed.

In accordance with this conception, vitamin-A deficiency states may be divided into groups, as follows :

1. Diseases due entirely or partially to degeneration of, or defects in, cell structure
2. Diseases due to failure in the maintenance of specialised non-structural proteins
3. Diseases due in the first instance to cell degeneration and resulting in invasion by pathogenic organisms to which

there is an impaired resistance as a result of lack of specialised proteins (antitoxins) for the formation of which vitamin A may be an essential factor

In the first group comparatively few conditions can be classified, since in deficiency states of such severity as to impair mucosal or epithelial integrity there is likely to be such a degree of antitoxin deficiency that subsequent bacterial infection is more or less inevitable. The most important, however, is perhaps phrynodermia (toad skin). Phrynodermia is among the classical symptoms of hypovitaminosis-A. It is generally preceded, however, by a number of other deficiency symptoms. Marrack has recently described these symptoms (*Brit. Journ. Indust. Med.*, April 1944, p. 114), and they are as follows :

Epithelial keratinisation is produced, and is often first seen in the conjunctiva (xerosis). The surface appears dull and wrinkled and later Bitot's spots appear. These are pearly-white patches about 2 mm. in diameter. In the more severe degrees of deficiency the cornea may ulcerate. The skin generally becomes dry and rough, round or oval papules may be formed, particularly on the front and sides of the thighs and back and sides of the forearms. These papules are caused by hyperkeratosis of the pilo-sebaceous follicles, and the condition constitutes phrynodermia.

This specific dermatosis of hypovitaminosis-A was first described by Frazier and Hu in 1930, and Youmans has described the lesions as follows : (" Nutritional Deficiencies ", Lippincott, 1941). The dermatosis " consists of a papular eruption usually occurring first on the anterolateral surface of the arms and thighs, spreading to involve the legs, abdomen, buttocks, back and neck. The lesions consist of dry papules of varying size up to 5 mm. in diameter, arising at the site of the pilo-sebaceous follicles, principally on the exterior surfaces of the thighs and arms but extending in some cases to the shoulders, abdomen, back, buttocks and, rarely, the face and neck. The papules are conical or hemispherical and contain a central intrafollicular plug which projects from the surface or is covered with a loosely adherent scale. When expressed the plugs leave gaping cavities. In mild cases the papules are no larger

than those of ordinary gooseflesh. The skin is generally dry, rough and wrinkled, darker than normal and often of a dull slate colour. There is an absence of sweating.

" In some patients the lesions present a somewhat different appearance. They consist solely of dull red, flat or slightly conical, discrete papules of varying size, usually about 0·5 cm. in diameter, similar to the lesion of acne. The papules are distributed over the anterolateral aspect of the arms, the shoulders, upper chest and back, with few, if any, on the face, abdomen, or lower extremities. The individual lesions often simulate a pustule, giving the impression that by piercing or removing the whitish top a drop of pus could be obtained. When such attempts are made, however, the cap is found to be a thin whitish scale which when removed leaves a raw surface and pus is not found. In some cases there are a few scattered pustules but comedones are uncommon. The skin does not appear to be dry or rough and itching does not occur. Microscopically the histologic changes are similar to the dry horny type, except for a slightly greater cellular reaction.

" The dermatosis is said to be uncommon in children, and Lowenthal related the increased evidence after puberty to the changes in the sebaceous glands and hair occurring at that time. Recent studies suggest that mild grades of the eruption are not uncommon and will be found if looked for carefully."

Hypovitaminosis-A may cause a keratinisation of the vaginal epithelium practically indistinguishable from that of œstrogen deficiency and senile vaginitis.

The rôle of vitamin A in the visual process has already been discussed in the section on the mode of action of vitamin A. Deficiency of the vitamin results in a slowing up of the process of regeneration of visual purple, with consequent delayed adjustment of the eyes to dim light after exposure to bright light. This delayed restoration of an essential retinal metabolite results in night-blindness, which may in extreme cases proceed to total night-blindness in which there is no recovery at all. The earlier stages of night-blindness frequently constitute the first symptoms of vitamin A deficiency.

Vitamin A has been given, somewhat empirically, for the treatment of gastric ulcer. Sixty per cent. of cases have been stated to have benefited from the treatment (*Wien. Klin.*

Wschr., July 9, 1938, quoted in the *Prescriber*, Feb. 1939, p. 45), but cases of duodenal ulcer did not respond.

Thirteen cases were treated with a vitamin A preparation (Avoleum) containing 30,000 international units per gramme. Disappearance of pain and discomfort as well as restoration of appetite were stated to be impressive and were noted in all cases (except one with " hour-glass " stomach) as early as seven days after the beginning of treatment (*Guy's Hospital Reports*), Vol. 90 (Vol. 20 Fourth Series), No. 1, 1940–41, p. 41). These findings have not been confirmed, and the treatment has now been more or less abandoned, possibly by reason, chiefly, of the restricted supplies of vitamin A available.

Daily Requirements of Vitamin A

Only approximate figures can be given for the daily requirements of vitamin A, as various circumstances are likely to cause variations in the amounts required by any individual. The following figures are therefore to be regarded as averages, which may be varied at the discretion of the physician.

	International Units
Infants (up to one year old)	2,000
Children (one year to 18 or 20 years)	2,500 to 8,000
Adults	5,000
Pregnant and lactating women	6,000
Average for all ages	5,000

Absolute deficiencies of dietary vitamin A are probably rare, so that doses of the same order as the daily requirement given daily will generally correct mild hypovitaminotic states in a short time. Generally, therefore, the daily requirements may be considered to be also the prophylactic doses of vitamin A.

Dosage of Vitamin A

Prophylactic

Doses of the order of the daily requirement, which may be summarised with sufficient accuracy for all clinical purposes, as follows :—

	International Units
Infants and young children	2,000 to 3,000
Adolescents	3,000 to 8,000
Adults	5,000
Pregnant and lactating women	6,000

L

Therapeutic

	International Units
Infants and children	15,000
Adolescents and adults	30,000 to 60,000

Hypervitaminosis-A

Doses of vitamin A far larger than the therapeutically effective doses suggested above can be given without producing any undesirable effects. Clinically, therefore, hypervitaminosis-A can be considered to be non-existent. Carotenæmia, already referred to, is rare and of no particular significance. The only symptom reported is the yellow, jaundiced appearance of the patient, which disappears on reducing the carotene intake to normal levels.

Toxic reactions have been reported following the ingestion of polar-bear liver, which appears to be the richest source of natural vitamin A so far discovered. Similar effects have also been reported following the eating of the liver of the seal *Phoca barbata*.

The reports have been summarised by Rodahl and Moore (*Biochem. Journ.*, July 1943, p. 166). All the members of an expedition to Novaya Zembla in 1596 became ill after eating polar-bear liver, and with three persons the illness was severe, with the loss of skin from head to foot. Inconsistent poisoning effects were reported by Kane in 1856. Polar-bear liver caused illness among members of an English expedition to Franz Josef Land (1894–97). Symptoms of toxicity may be expected to appear two to four hours after ingestion of the liver, and may include drowsiness, sluggishness, irritability or an irresistible desire to sleep, severe headache and vomiting. After twenty-four hours the skin may begin to peel, starting in spots around the mouth and spreading to larger areas. The peeling may be confined to the face, or it may become general. In rats, enteritis, emaciation, pneumonia, seborrhœa and alopecia have been described. One rat which ate 33·1 grammes of liver during 22 days (an average of 15,000 international units daily) became anæmic, and the hind legs were paralysed. It was killed when moribund, and typical profuse internal hæmorrhage was found. This was particularly evident under the skin and pericardially.

It is suggested that 100,000 international units of vitamin A may be expected to cause immediate illness in a rat, and that this is equivalent to about 7,500,000 international units for a 70-kilo man.

Acute illness has been reported following the ingestion of 4 to 5 oz. of halibut-liver oil for five days, representing a daily dose of about 6,000,000 units.

Rodahl and Moore conclude that polar-bear liver is toxic by reason of its high content of vitamin A (perhaps 20,000 units per gramme), but they do not exclude the possibility of other toxic substances being present.

The opportunity or inclination to ingest toxic doses of vitamin A must be rare under normal conditions, and hypervitaminosis-A may therefore be considered to be highly improbable from the clinical point of view.

Excesses of vitamin A over normal requirements are for the most part destroyed in the Kupfer cells in the liver, and little if any of the vitamin is excreted.

Supplementary Note on "Vitamin F"

"Vitamin F" is an obsolete name for vitamin B_1 in the U.S.A. In Britain the name was applied to a postulated fat-soluble factor supposed to be necessary for maintaining a healthy state of the skin and possibly of such of its appendages as hair and nails. A particularly high concentration of the factor has been reported as occurring in linseed oil.

"Vitamin F" no longer retains its status as a vitamin, but is regarded as being an essential dietary factor. Its precise identity has not been established, but it is known to be a highly unsaturated fatty acid, possibly linoleic, linolenic or arachidonic acid, or a mixture of these. Phytadienic acid has similar properties.

Since the recognition of "vitamin F" as a non-vitamin dietary factor, a paper on its importance as a nutrient in maintaining a healthy state of the skin has appeared in the *Medical Press and Circular* (May 26, 1943, p. 331).

CHAPTER XVII

VITAMIN D

THE early history of vitamin D is bound up with that of vitamin A, the original " fat-soluble A " factor which was thought to be one substance, but later proved to contain both an antixerophthalmic and an antirachitic factor. The recognition of this fact resulted from work carried out on " fat-soluble A " which has been briefly surveyed in the opening paragraph in the previous chapter (page 148).

The existence of the two factors in " fat-soluble A " was confirmed from evidence which was gradually accumulated between 1923 and 1925, and which showed that ultra-violet irradiation of certain foodstuffs or the animal body produced the antirachitic effect but not the antixerophthalmic effect.

Hess and Weinstock (1924 and 1925) found that it is fats which become antirachitic after ultra-violet irradiation and that they lose this property if the irradiation is prolonged unduly. In the year 1925 three groups of investigators (Steenbock and Black; Hess, Weinstock and Helman; Rosenheim and Webster) discovered independently and simultaneously that the substance in fats susceptible to the effects of irradiation occurred in the unsaponifiable fraction, and they were of the opinion that it was cholesterol. Cholesterol purified by recrystallisation more than twenty times retained the property of becoming activated.

It was subsequently noted by Rosenheim and Webster (1926), however, that more than 99 per cent. of the cholesterol remained unchanged after irradiation. Precipitation of the unchanged cholesterol from solution with digitonin left the active substance in solution from which it could subsequently be recovered. Rosenheim and Webster also noted that ergosterol (obtained from ergot) became highly antirachitic when irradiated. A brief period of intensive investigation by several groups of workers followed these findings, and in 1927 it was announced jointly by Rosenheim and Webster on the one hand and by Windaus and Hess on the other that

ergosterol and the provitamin-D impurity in cholesterol were one and the same thing.

Although a highly potent antirachitic substance could now be prepared by the ultra-violet irradiation of purified ergosterol, it was still not possible to produce pure vitamin D. Irradiated ergosterol is a mixture of at least six substances, resinous in nature and steadfastly resistant to crystallisation. Vacuum distillation with fractional condensation was employed in 1930 by Bourdillon, Askew *et al.* to separate the mixture, and they introduced the name " calciferol " for the active antirachitic which they succeeded in isolating.

The other irradiation products of ergosterol are lumisterol, tachysterol, toxisterol and suprasterols I and II. None of these has antirachitic properties and most of them are toxic. From this mixture Windaus isolated " vitamin D_1" before Bourdillon and Askew isolated calciferol. Subsequently " vitamin D_1 " proved to be a molecular compound of calciferol and lumisterol.

For a long period it was thought that calciferol was the true natural vitamin D of animals, produced by the action of ultra-violet light on ergosterol in the skin or on the fur or feathers and subsequently ingested during " washing " in cats and rabbits and during preening in birds. Ergosterol is the sterol characteristic of the lower plants (indeed, it was so named because it was isolated from ergot) and occurs only in minute amounts, if at all, in animals. Further, ergosterol itself is not absorbed from the intestinal tract.

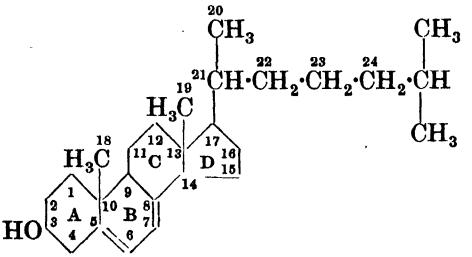
Subsequent work revealed that the vitamin D of cod-liver oil, and from other animal sources, was an irradiation product of 7-dehydrocholesterol, and it came to be known as vitamin D_3. One of the facts which led to the suspicion that calciferol was not the so-called " natural " vitamin was that califerol is very much less antirachitic for chicks than vitamin D_3.

Chemistry of Vitamin D

It is now known that many sterols, on irradiation, produce antirachitic substances. These substances for the most part have the same sterol nucleus as ergosterol, but differ from it in the side-chain attached to carbon atom 17. Cholesterol, in a sense a parent substance of vitamin D_3, is of very consider-

able importance in various biochemical processes in animal physiology. It appears to be a parent substance of several of the substances acting as vitamin D and, in addition, the steroid hormones of the gonads and of the suprarenal cortex are probably elaborated from it in the animal body. It is apparently involved in the metabolism of fats, and blood-cholesterol levels are significant as indicators of the state of thyroid activity.

Cholesterol is a characteristic sterol and has the structure indicated below :

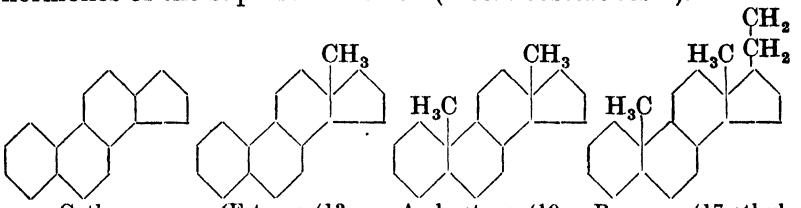


The lettering of the rings and the numbering of the carbon atoms indicated in this structural formula are those generally employed in the descriptive chemical names of the steroids and in specifying particular parts of the molecules. They are therefore worthy of consideration in some detail here, for an understanding of this numbering system simplifies the understanding of the chemistry of all the sterols.

The condensed-ring system constituted by the rings A, B and C will be recognised as phenanthrene, fully hydrogenated to form perhydrophenanthrene. The five-membered ring, D, is *cyclo*pentane, and condensed with polyhydrophenanthrene this forms *cyclo*pentanoperhydrophenanthrene, the saturated hydrocarbon parent substance of all sterols. The short name " cythrene " has been suggested (*Journ. Amer. Pharm. Assoc.*, Nov. 1943, p. 274) for *cyclo*pentanoperhydrophenanthrene.

From cythrene, a number of other saturated hydrocarbons (" cyclic paraffins ") may be regarded as being derived. Substitution of a methyl group for the hydrogen on carbon atom 13 (C_{13}) gives œstrane, the parent substance of the natural œstrogens. If the hydrogens on both C_{13} and C_{10} are replaced by methyl groups, androstane is formed, the parent hydrocarbon of the androgenic hormones. Substitution of one of the

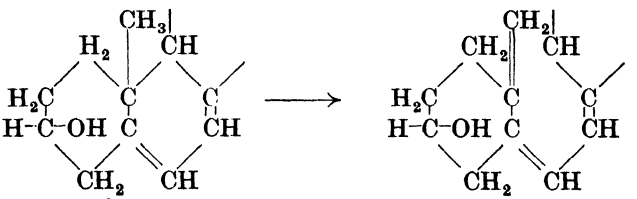
hydrogen atoms on C_{17} of androstane with an ethyl group gives pregnane, parent hydrocarbon of the progestational (corpus luteum) hormone, the " provitamins-D " and the hormones of the suprarenal cortex (" corticosterones ").



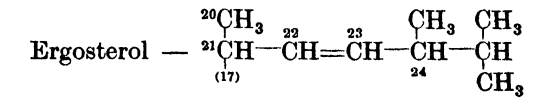
Cythrene — Œstrane (13-methylcythrene) — Androstane (10 : 13-dimethylcythrene) — Pregnane (17-ethyl-10 : 13-dimethylcythrene)

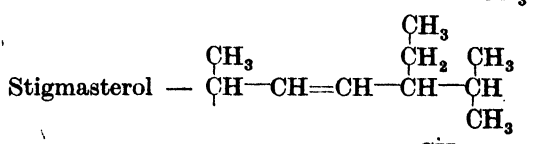
The " provitamins-D " are derivatives of pregnane in which a long side-chain replaces one of the hydrogens on C_{21} (as in cholesterol) and from which one or more hydrogen atoms are removed from ring A or B. Generally it is one or both of carbon atoms 5 and 7 (ring B) which are dehydrogenated. Both are dehydrogenated in ergosterol and in cholesterol.

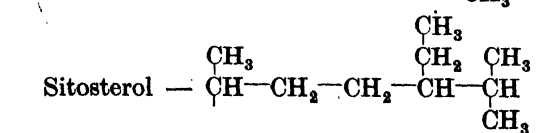
The effect of ultra-violet irradiation on a provitamin D is the opening out of ring B as indicated below :—



The important side-chains on C_{17} are :—







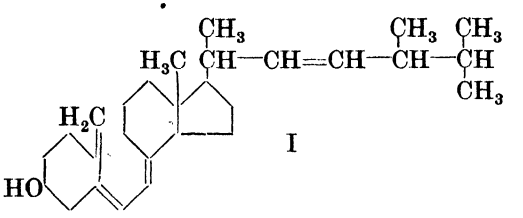
Cholesterol — (see above, page 166)

Sitosterol is the principal constituent on the unsaponifiable fraction of wheat-germ oil, the chief source of vitamin E.

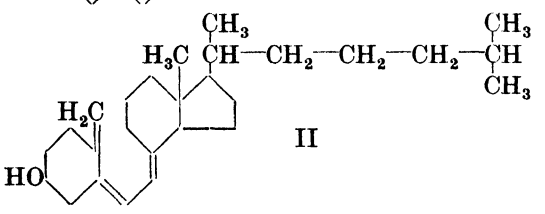
Of the various forms of vitamin D, two only need be considered here: calciferol, the so-called artificial vitamin and the natural substance, vitamin D_3, of fish and other animal oils.

Calciferol

Calciferol is formed from ergosterol as indicated above (page 166). It has the structural formula shown below (I). The formula of vitamin D_3 is given (II) for comparison.



I



II

Chemically, calciferol has been described as 9/10-ergostatetraene-(18 : 10, 5 : 6, 7 : 8, 22 : 23)-ol-3. Reference to its structural formula will reveal the full meaning of this name.

If protected from ultra-violet light, calciferol is reasonably stable, but the vitamin itself should be stored *in vacuo* or in an inert atmosphere in a cool place. Solutions of calciferol in oil stored under reasonable conditions probably lose little of their activity in periods of not more than a year. There appears to be some reason, however, to suspect that preparations containing calciferol and a calcium salt may lose their activity somewhat more quickly.

There is no simple chemical test for calciferol. The B.P. (Addendum, 1936) gives a test for identity which is the determination of the melting point and the specific rotation of the 3 : 5-dinitrobenzoate of calciferol and a test for purity

which merely demonstrates the absence of ergosterol. Two biological methods of essay are given in the B.P. (1932) itself. The first is based on a comparison of the healing effect of a known amount of the standard preparation of calciferol and a sample of the unknown on rickets in rats (the " line " test). The comparison is made by examining the distal ends of the radii and ulnæ of rats from each group after staining with silver nitrate. In the second method the comparison is made in terms of the ash content of the bones of rats from each group which have been given the vitamin after a period of feeding with a rachitogenic diet.

Vitamin D_3

Vitamin D_3, the so-called " natural " vitamin D, irradiation product of 7-dehydrocholesterol, has been synthesised, but this is not generally carried out commercially, nor is the naturally occurring substance normally isolated. Cod-liver oil provides this vitamin and is sometimes specially recommended because it is suggested that the oil contains valuable " accessory factors " in addition to the vitamin A. This is not certain, however, and any beneficial effects attributed to such hypothetical factors may be the result of the intake of extra fat which the taking of cod-liver oil in effective doses involves.

Cod-liver oil is by no means the richest source of either vitamin D or of vitamin A. It usually contains these two vitamins, however, in the proportions which have come to be regarded in some measure as the " natural " proportions—that is, 10 international units of vitamin A to 1 international unit of vitamin D.

Halibut-liver oil is a rich source of vitamin A, but contains only a relatively insignificant amount of vitamin D.

Pharmaceutical Preparations of Vitamin D

Numerous preparations containing vitamin D are described in the British Pharmacopœia and its Addenda, to which reference should be made. Not all of the official preparations need be described here. They include cod- and halibut-liver oils providing vitamin D_3 and various preparations containing calciferol.

In the Pharmacopœia itself (1932) the official preparation was solution of irradiated ergosterol. Irradiated ergosterol is essentially a mixture of the irradiation products of ergosterol, including calciferol, but so prepared (by suitable choice of solvent and control of the time of irradiation) as to reduce the content of toxic substances to a minimum. Solution of irradiated ergosterol is therefore practically identical with viosterol, the official preparation in the United States of America. Both products are somewhat toxic, and the British preparation has been superseded by solution of calciferol (B.P., Addendum, 1936). All three of these products have the same antirachitic potency (3,000 international units per gramme).

A preparation of pure calciferol, described as " electrically activated " ergosterol, is issued in the U.S.A. under the proprietary name " Ertron ". This has been recommended for the treatment of arthritis. The exceptionally large doses required (400,000 to 600,000 international units daily) make the use of a purer preparation than viosterol essential. Specially strong solutions of calciferol are comparable to " Ertron " in therapeutic effect and, like " Ertron ", free from toxic effects apart from those inseparable from pure calciferol in large doses. " Ertron " has created a certain amount of lay and medical interest in this country, although it is not available here. Calciferol may be administered (with suitable precautions which will be mentioned in a subsequent section) in order to produce the therapeutic effects of " Ertron ".

Administration of Vitamin D

Vitamin D is administered, almost invariably, by the oral route. It is unchanged in the acid secretions of the stomach and absorbed with the fats and fat-soluble substances from the intestine. Thus, in the absence of marked vomiting, there is no hindrance to its absorption except in patients with cœliac disease or steatorrhœa or who habitually take large doses of liquid paraffin, in which vitamin D, being soluble, is excreted unabsorbed. Even when the patient has cœliac disease, however, adequate amounts of the vitamin will be absorbed from preparations such as solution of calciferol B.P. or preparations containing at least an equal concentration of the vitamin in solution in oil.

Unit and Potency of Vitamin D

The international unit of vitamin D is the antirachitic potency of $\frac{1}{40000}$ mg. of the international standard preparation of calciferol.

Although for some animals calciferol is relatively inert, for humans, calciferol and vitamin D_3 are equally potent (*Lancet*, May 25, 1940, p. 961).

Mode of Action of Vitamin D

Even less is known of the mode of action of vitamin D than of the mode of action of vitamin A. Apart from its function in promoting the absorption of calcium and phosphorus, even its functions in the body still remain somewhat obscure.

It has been observed that the fæces of rachitic rats are slightly alkaline, but that when vitamin D is administered in curative doses, there is a change to the slightly acid side. This is attributed to the action of vitamin D, and is perhaps the main factor in promoting the absorption of calcium and phosphorus. It is interesting to compare the action of the vitamin with that of another sterol, the ovarian hormone, in controlling the hydrogen-ion concentration of the vaginal secretion.

It seems possible that the fundamental action of vitamin D is on phosphorus and that its action on calcium metabolism is secondary to and dependent on this action on phosphorus. Vitamin D, it appears, is an essential factor for the formation of inorganic phosphorus compounds in the body from organic phosphorus compounds in the bones, serum and other tissues; that is, it is in a sense a " co-phosphatase ". An analogous effect to the phosphorylation of fats may take place in the absorption of calcium from the intestine, vitamin D being essential for the mobilisation of the necessary inorganic phosphorus. The ability of the osteoblastic cells to utilise calcium and phosphorus depends on the level of these substances in the serum and in the fluids bathing the cells. This level is controlled in some unexplained manner by vitamin D, probably in conjunction with other substances, particularly bone phosphatase.

Vitamin D may also play a part in the phosphorus metabolism of muscle tissue.

Although it has been suggested that vitamin D produces its effects in the body indirectly by acting on the parathyroid glands, this appears to be unlikely, since the effects of the hormone and of the vitamin are different.

Parathyroid hormone in excessive amounts causes de-calcification of bone and replacement with fibrous tissue and giant cells. Excessive amounts of the vitamin cause dissolution of the trabeculæ and no fibrous replacement. Further, rickets is made worse by parathyroid hormone, which raises blood calcium by mobilising calcium from bones, whereas vitamin D promotes absorption of calcium, raises its level in the tissue fluids and so promotes calcification of bone. There is a possibility that parathyroid hormone promotes the urinary elimination of phosphorus under certain circumstances: Possibly it acts with vitamin D or against it under different conditions, tending in either case to maintain an optimum calcium/phosphorus ratio in the body.

The mode of action of vitamin D in large doses in the treatment of arthritis is entirely unknown—indeed, its value is questioned—and this application of the vitamin is not accepted by the Council on Pharmacy and Chemistry of the American Medical Association.

Clinical Uses of Vitamin D

The only established indications for vitamin D are those conditions attributable to deficiency of calcium due to lack of the vitamin and consequent malabsorption. As a result of the somewhat limited distribution of vitamin D in the common articles of diet, as well as of the limited irradiation of the body through lack of sunshine and the screening effect of clothes, vitamin-D deficiency is fairly common. In adults, in whom the daily requirement is low, this is usually not a serious matter. But in infants and children, whose calcium requirements are high, there is a correspondingly high requirement of vitamin D. Thus an extra-dietary source of vitamin D (and often of calcium) is essential not only from birth to the end of the age of growth, but also before birth. Therefore calcium and vitamin D should be given during pregnancy, so that the reserves of the mother

are not drawn upon by the fœtus to such an extent as to produce a hypocalcæmic condition.

Frank rickets is now fortunately a rare condition, but minor degrees of deficiency are by no means uncommon. Bone deformities, particularly of the legs, are perhaps the most common symptoms of relatively marked deficiency of vitamin D. Such deformities can be corrected simply by the administration of vitamin D if treatment is begun in the early stages and if adequate amounts of calcium are absorbed at the same time. In adults, vitamin D is indicated for the treatment of osteomalacia and spasmophilia. It has been recommended for use in psoriasis and in arthritis, but its value in these conditions is questioned and as yet unconfirmed. In arthritis the postulated effect of vitamin D cannot be a vitamin effect, but is probably entirely pharmacological. Similarly some non-vitamin properties of vitamin are probably utilised when it is given for the treatment of acne vulgaris and asthma (with allergic symptoms of extrinsic origin).

Prophylactically, vitamin D should be administered as a routine to women during pregnancy, to infants and young children and (in a potent or fat-free form) to all patients whose absorption of fats and fat-soluble substances is impaired. These last include patients with cœliac disease, steatorrhœa and sprue.

Daily Requirement of Vitamin D

The precise amounts of vitamin D required to maintain normal health are very imperfectly known. Many factors appear to play a part in modifying the amounts required by different individuals and by one individual under different circumstances. The requirement is undoubtedly highest during the periods of growth, and will be directly proportional to the calcium requirements. Adults certainly require less than infants and children in relation to body-weight and possibly also absolutely, except of course during pregnancy, when the mother's intake must be sufficient to provide for the needs of the developing fœtus and to meet her own needs, which may be increased to some extent in consequence of her increased metabolism during pregnancy and lactation.

Dosage of Vitamin D

Prophylactic. In order to overcome dietary deficiencies of vitamin D, one or other of the numerous preparations of the vitamin should be given in doses which will provide the daily requirement of the individual (see p. 173). An adequate intake will thus be insured.

The Addendum, 1936, to the B.P., 1932, gives a prophylactic dose of 1,000 to 2,000 international units of calciferol daily for an infant—equivalent to 5 to 10 minims of solution of calciferol B.P. (10 to 20 drops approximately).

Therapeutic. A wide range of doses of vitamin D has been suggested in various conditions, and these are most conveniently given in tabular form :—

Condition	Dose in International units	Dose in convenient Pharmaceutical Preparation
Rickets, Dental caries, Minor skeletal defects (at all ages)	1,000 to 3,000 daily	10 to 20 drops of liquor. calciferol. B.P.
Osteomalacia, Infantile tetany, Cœliac disease, Spasmophilia	6,000 to 12,000 daily	½ to 1 teaspoonful of liquor. calciferol. B.P.
Bone fractures (to hasten healing of)	1,500 to 3,000 daily	15 to 30 drops of liquor. calciferol. B.P.
Acne vulgaris	1,500 daily	15 drops of liquor. calciferol. B.P.
Arthritis (patient under close medical observation)	200,000 to 600,000 daily	Solutions containing 100,000 or 200,000 units per grm. are available. Doses should be accurately measured.

Hypervitaminosis-D

Toxic reactions produced during the clinical use of solution of irradiated ergosterol gave rise to the impression that there was considerable risk of producing undesirable and even dangerous reactions if the vitamin was given in more than minimum doses. As has already been indicated, the toxic effects produced were attributable for the most part to the non-antirachitic products of irradiation of ergosterol.

When pure calciferol came into general use, toxic reactions became much less common. The fact remains, however, that toxic reactions are probably more easily produced from the administration of vitamin D than from the administration of any other vitamin. Comparatively small doses of calciferol (3,000 to 5,000 units daily) if repeated for several days may cause slight headache and a " liverish " feeling in some persons, particularly if they are not in a state of vitamin D deficiency, and perhaps more commonly in adults than in children. Marked symptoms are not usually produced, however, unless doses of several hundred thousand units are given. The symptoms then produced are chiefly headache, lassitude, nausea, vomiting, diuresis, diarrhœa, muscular atony and, occasionally, severe depression. It is unlikely that such symptoms will be encountered unless an excessive dose of the vitamin is taken inadvertently or during the treatment of arthritis with large doses.

Recently it has been stated that calciferol in doses of 50,000 international units once to three times daily over a considerable period is of marked value for the treatment of lupus vulgaris. It may also prove to be of value in tuberculous adenitis.

CHAPTER XVIII

VITAMIN E

IT was for long thought that a diet which was sufficient to maintain growth and good health was also sufficient to maintain the reproductive function. Mattill and Conklin in 1920, however, observed that rats reared on whole milk grew well and appeared to be in good health, but were sterile. Evans and Scott (1920) postulated an unknown substance, X, necessary for fertility and which occurred in fresh lettuce leaves, wheat embryo and dried alfalfa. During the next year Evans and Bishop showed that this factor X was fat-soluble and not identical with any of the known vitamins. Sure confirmed these findings in 1923 and suggested that this factor X should be called vitamin E, a suggestion which was generally accepted two years later.

For a considerable period it was thought that vitamin E was essential for fertility in the human female, as it is in the rat. This has never been confirmed, however, and is not now generally accepted.

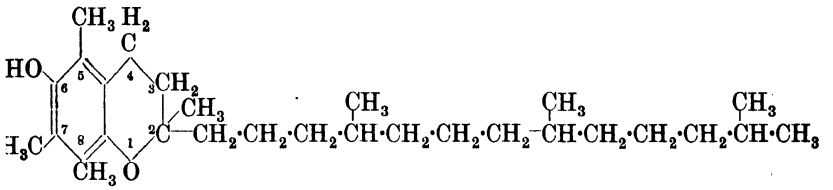
The form of sterility in female rats on an E-deficient diet consists of the death and resorption of fœtuses after an apparently normal pregnancy. This may perhaps be more correctly interpreted as an analogous condition to habitual abortion in humans rather than as analogous to human sterility. This point will be more extensively discussed, however, in a subsequent section.

Chemistry of Vitamin E

The difficulties of fractionating the complex mixture of substances in wheat-germ oil (one of the richest natural sources of vitamin E) and the slowness and difficulties of the biological test for the vitamin long delayed the isolation of vitamin E and the determination of its chemical structure. Evans, Emerson and Emerson were able, in 1936, to isolate the allophanates of two alcohols, α- and β-tocopherols, in crystalline form. α-Tocopherol was found to be $C_{29}H_{50}O_2$, and β-tocopherol

is a lower homologue with the composition $C_{28}H_{48}O_2$. Subsequently an isomer of β-tocopherol was isolated from cotton-seed-oil, and this was named γ-tocopherol. (The name "tocopherol" was derived from the Greek words, τόκος, birth or offspring, and φέρω, to bear.)

The precise structure of α-tocopherol was determined by Fernholz (*Journ. Amer. Chem. Soc.*, 1938, **60**, 700) and is represented by the following structural formula :—



that is, 5 : 7 : 8-trimethyltocol.

β-Tocopherol and γ-tocopherol are the 5 : 8- and 7 : 8-dimethyltocols respectively. The trimethyl derivative (α-tocopherol) is the most active of the three compounds, but all three generally occur together. All three of the "vitamins E" are readily oxidised and inactivated in air and by ultra-violet light even in the absence of air. Oxidation is accelerated by traces of iron.

Unit and Potency of Vitamin E

Esters of the vitamin are considerably more stable than the free vitamin, and α-tocopheryl acetate has been adopted as the international standard preparation. The international unit of vitamin E is the activity of 1 mg.

Preparations of vitamin E are normally estimated biologically, using avitaminotic pregnant rats.

Four chemical and physical methods of estimation have been suggested, but they do not appear to have been generally adopted. The methods are :—

i. Spectroscopic—estimation of intensity of absorption at 294 mμ.

ii. Electrometric—titration with gold chloride.

iii. Colorimetric—oxidation with ferric chloride and estimation with α : α′-dipyridyl of the ferrous compound so formed.

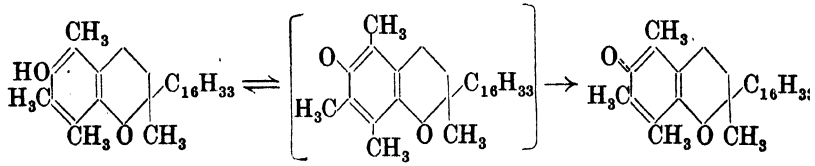
iv. Colorimetric—estimation of the red colour produced with nitric acid.

None of these methods, it will be seen, is suitable for ordinary use by pharmacists or physicians. A description of the $\alpha : \alpha'$-dipyridyl method is given in Allport's "Colorimetric Analysis" (Chapman and Hall, 1945), p. 371.

In consequence of the ease with which vitamin E is inactivated, preparations containing it must be stored and dispensed so as to protect it from exposure to air and sunlight and from contact with iron. Solutions in oil will be inactivated by oxidation before oxidation has proceeded so far as to turn the oil rancid.

Physiological Action of Vitamin E

The mode of action of vitamin E has probably given rise to more speculation and to more unconsidered dogmatic statements than has the mode of action of any other vitamin. Its ready oxidisability might suggest that it plays a part in some oxidation-reduction system, but the inactivity of the oxidised vitamin appears to make this suggestion unlikely. There is one oxidation process of α-tocopherol, however, which may prove to be of some biological significance (*Journ. Biol. Chem.*, 1940, **134**, 536) :—



(Phenoxyl radicle) α-Tocopherylquinone

Further, the tocopherols appear to act as inhibitors of the autoxidation of the vegetable oils in which they occur naturally (*Pharm. Journ.*, Oct. 30, 1943, p. 163). Quite definite optimum concentrations of tocopherols are necessary for the manifestation of these autoxidation-inhibiting properties. The addition of α-tocopherol to a vegetable oil raises the concentration of the vitamin above the optimum level, and the tocopherols become inert. At least three types of inhibitors of one class act "synergistically" with inhibitors from another class. As an example, tocopherols on oxidation give tocoquinones, which

in turn are reduced to tocohydroquinones. In the presence of a mineral acid such as phosphoric acid, the tocohydroquinones are cyclised, forming tocopherols once more. Certain organic acids also exert the same effect.

Hickman, Harris and Woodside have produced evidence which appears to show that vitamin E acts *in vivo* as an anti-oxidant to vitamin E (*Nature*, July 18, 1942, p. 91). They even conclude that the first function of vitamin E is to preserve the vitamin A before, and perhaps during, intestinal absorption. Preservation of vitamin A at the site of its action is postulated as a secondary function. Vitamin-E deficiency, it is suggested, may result in a reduction of blood-carotene level in pregnant rats to a level below that compatible with life. Their suggestion that deficiency of either of these vitamins may result in the appearance of deficiency symptoms of the other is in agreement with the suggestion of Davies and Moore (*Nature*, June 28, 1941, p. 794) that whitening of rats' teeth previously attributed to deficiency of vitamin A may indicate a deficiency of either vitamin A or E. Deficiency of vitamin E, however, produces an unusual type of atrophy of the enamel organ in the teeth of rats receiving adequate vitamin A (*Nature*, July 25, 1942, p. 122).

By reason of the extremely limited knowledge of the chemistry of the action of vitamin E, the site (or sites) of action of the vitamin is also a matter of considerable discussion. There are two main theories, however, which are deserving of consideration in some detail. Briefly these theories are :—

1. That vitamin is required generally by all body tissues, but that certain tissues may have particularly high requirements.
2. That vitamin E is required only by a few specialised tissues, perhaps only the anterior lobe of the pituitary gland or the gonads or possibly both.

It should be stated at this point that there is some evidence for believing that all species do not appear to need vitamin E. Goats, for example, appear to be quite independent of vitamin E in the diet. It has even been questioned whether it is necessary for man. This doubt should at least have the effect of counteracting any tendency to over-estimate the importance

of vitamin E in man or any other species, with the possible exception of rodents, particularly rats.

Theory of General Tissue Requirement of Vitamin E (1)

This theory, or perhaps group of similar theories, appears to be more generally supported in the U.S.A. than in Great Britain. In particular, the work of Adamstone and of Mason is in agreement with a theory of the general tissue requirement, and it receives the support of Drummond in England.

A general cellular oxidation–reduction process (quinol-quinone system) has been suggested, but isolated tissues from E-hypovitaminotic animals show no decrease in oxygen uptake. Apart from its specific action in the reproductive process, probably via the pituitary and gonads, vitamin E deficiency disturbs the development of the hæmopoietic system at a very early stage of embryonic development, probably interferes with the formation of chromatin, retards growth in early adult life and gives rise to disorders of the nervous system and degenerative changes in the muscles in old age (Drummond). In rats, vitamin-E deficiency produces other symptoms which are indicative of a generalised action of the vitamin as distinct from its action on the reproductive glands (anterior pituitary and gonads). These symptoms are :—

1. Deposition of small yellow granules in the muscular layers of the uterus and seminal vesicles giving a brown discoloration.
2. Paralysis of the hind limbs with slight discoloration of the affected muscles.
3. Degeneration of the convoluted tubules of the kidneys; the interstitial tissues and glomeruli are unaffected.
4. Skin sores, emaciation and death.

To these manifestations of vitamin-E deficiency must be added the effects on the vitamin-A metabolism of rats. It has been stated that on an ample diet sufficient vitamin A can be stored in the liver to last 100 years. Depletion of such a store is particularly rapid unless the intake of vitamin E is adequate. Indeed, vitamin E deficiency may also cause an excessively rapid elimination of vitamin A, as shown by an unnatural

whitening of rats' teeth on a diet deficient in either of these vitamins (*Nature*, June 26, 1941, p. 794, previously mentioned). In chicks, vitamin E deficiency has been said to give rise to abnormal permeability of capillaries and a consequent " alimentary exudative diathesis ".

Generally, it has been suggested that vitamin E may be concerned in the maintenance of normal brain or nerve tissue, deficiency in rats resulting in an encephalomalacia accompanied by absence of the normal increase in brain cholesterol. There appears to be a relationship also between the vitamin E and the utilisation of a number of steroids and related substances, vitamin D, androgens, œstrogens, and some carcinogenic compounds. Creatine–creatinine metabolism is certainly dependent upon vitamin E, a fact which has led to the use of the vitamin in myasthenia gravis, although with somewhat disappointing results. The reason for this absence of effect is obscure, particularly as theoretical considerations appear to indicate that it should be of some value. A characteristic symptom of both myasthenia gravis and of hypovitaminosis-E is creatinuria. It would appear that this indicates deficient utilisation of the creatine, the phosphate of which is a phosphate donor for the phosphorylation of hexose and for the activation of muscle protein on which muscle contraction depends. Thus the ætiology of myasthenia gravis remains obscure, although there appears to be an interesting relationship between the disease and the thymus gland, which is discussed in the chapter on the thymus.

Confirmation of the general requirement of all tissues of the body for vitamin E must await a detailed description of its mode of action. Tissues in which metabolic processes are specially active will, it is reasonable to assume, require relatively large amounts of vitamin E. This appears to be especially true of tissues undergoing structural modification and growth (protein metabolism) and the anterior pituitary gland, which is continuously secreting protein or polypeptide hormones. The relationship between the thymus gland and vitamin E may also be significant in this connection, for it is possible that the thymus hormone is a polypeptide which is of importance in inhibiting the appearance of sexual maturity. Other examples of the association of vitamin E with more than

average metabolic rates in specialised tissues include its direct or indirect action on endometrial and breast tissue (the vitamin appears to potentiate the action of progestin) and the as yet inadequately understood association between vitamin E and muscle metabolism. Further, the action of the vitamin on the dental tissues of rats already discussed may be another example.

The anterior lobe of the pituitary gland undoubtedly requires relatively large amounts of vitamin E, as has been shown by Barrie in a series of papers. The following are particularly important, and the original papers should be consulted if possible :—

Lancet, July 31, 1937, p. 251.
Biochem. Journ., 1938, Vol. XXXII, No. 9, p. 1467.
Ibid., p. 1474.
Ibid., 1938, No. 12, p. 2134.
Journ. Obst. and Gynæc. Brit. Emp., Feb. 1939, p. 49.

A considerable body of evidence is given in these and other papers which, briefly, Barrie interprets as indicating that the actions of vitamin E are exerted mainly through its action on the anterior lobe of the pituitary gland. This evidence appears to constitute the bulk of that supporting the theory first enunciated by Verzar (*Arch. ges. Physiol.*, 1931, **227**, 499).

Theory of Specific Action on Anterior Pituitary (2)

Verzar (*ibid.*) suggested that the effects of hypovitaminosis-E are comparable with those of hypophysectomy. On the other hand, administration of active anterior pituitary extracts generally fails to correct the effects of vitamin E deficiency. It is possible, however, that this subject requires further investigation.

The antiabortive effect (or, in rats, the " antifœtus-resorption " effect) of vitamin E is attributed in the " specific " theory to its effect on the anterior lobe of the pituitary and consequent indirect effect on ovaries, placenta and fœtus. There is little doubt that there is such an action, manifested principally, perhaps, in the curative effect of vitamin E in cases of habitual abortion. A more direct effect of vitamin E must be postulated, however, to account for the relatively

rapid effect of the vitamin in threatened abortion. If it is accepted (as has been reported) that vitamin E is ineffective in threatened abortion if it is given after placentation has normally been completed, it is still necessary to postulate a direct action of vitamin E in order to account for the relative rapidity of its action in vulvo-vaginitis as reported by Shute. But this action of the vitamin perhaps belongs to the " general " rather than the " specific " theory of its action.

Barrie's work has shown that a number of the results of hypovitaminosis-E are accounted for by the " specific " theory. For example, deficient lactation and hypothyroidism are attributable to deficient secretion of galactotropic and thyrotropic hormones. But, as Vogt-Møller has stated, the order of the appearance of lesions in pregnant rats—fœtus, fœtal placenta, maternal placenta—argues " against a definite relation (presumably through the pituitary) between vitamin E and progesterone, unless we accept the hypothesis that this hormone exerts a specific influence upon the development of the ovum."

There is, therefore, perhaps rather more than a strong probability that vitamin E exerts a direct action in abortion (or in fœtal absorption in rats) which is at least as important as its indirect action through the pituitary.

Shute's Antiœstrogen Theory of Vitamin-E Activity

No attempt is made by Shute to explain the whole of the physiological properties of vitamin E in terms of this theory, which is not incompatible with either the " general " or " specific " theory. It does, however, explain certain actions of the vitamin which are not otherwise explained, and is worthy of some consideration here.

Grafenburg in 1909, Abderhalden in 1914 and Blair-Bell in 1929 all suggested some resistance to the normal proteolytic penetration of the placental villi as a cause of partial or complete detachment of the placenta and consequently of uterine hæmorrhage or abortion. This resistance to proteolysis was observed in E-deficient rats, and Shute postulated that vitamin E inhibits an œstrogenic substance in normal animals (or humans), whereas in cases of vitamin-E deficiency the œstrogen is free to inhibit the normal proteolytic action of the placental villi in penetrating the endometrial tissues of the mother,

an essential condition for consolidating the attachment of the placenta.

Shute has gone farther in his theory of the antiœstrogenic action of vitamin E. Certain cases of senile vulvo-vaginitis are made worse by the usual treatment with an œstrogen, and Shute maintains that such cases are due to excessive secretion of œstrogen and that the logical and effective treatment is the administration of vitamin E.

He claims successful and remarkably rapid effects with this form of treatment.

An antiœstrogenic effect might be exerted by vitamin E directly, but since the vitamin tends to stimulate anterior pituitary activity, it is possibly an indirect antiœstrogen. However, such an effect is likely to be more delayed in appearing than Shute's clinical results with the treatment of vulvo-vaginitis indicate. It is probable, therefore, that vitamin E is a direct antiœstrogen and likely to augment the action of progestin, the physiological antagonist of the œstrogens.

Shute's theory is thus more in accordance with the " general " rather than with the " specific " theory of vitamin-E action.

The functional association of vitamins A and E has already been mentioned and both vitamins have been associated with protein metabolism. Shute's theory of the antiœstrogenic function of vitamin E, particularly the postulated effect of vitamin E in counteracting the effect of œstrogenic hormones and so allowing the normal proteolytic (trypsin) action of the villi to proceed and a firm placental attachment to be formed provides a further association with protein metabolism.

Another point, not previously mentioned, is of interest in this connection, although its significance is as yet somewhat obscure. Vitamin E deficiency in female rats invariably results in a brown discoloration of the muscular layers of the uterus, due to the deposition of small yellow granules. In male rats the seminal vesicles become discoloured. If the deficiency is prolonged until the muscular degeneration described by Ringsted appears, slight discoloration of the muscles of the hind limbs appears just before the muscular dystrophy becomes obvious (Moore, Martin and Rajagopal). Barrie states (*Proc. Roy. Soc. Med.*, June 1939) that this uterine

pigmentation results from feeding rats with a diet completely lacking in vitamin E. Administration of vitamin E has no effect in eliminating or even reducing this pigmentation unless the administration is followed by gestation. The increased blood supply to the uterus during pregnancy is presumably responsible for the elimination of the pigment.

The precise nature of the pigment has not been determined, but a preliminary examination of it has been made. It is an amorphous, acidic, brownish-yellow powder which in fluorescence and solubility resembles the " melanins " formed by acid hydrolysis of proteins (*Biochem. Journ.*, April 1943, Proceedings, p. 2). It is probably an iron-free decomposition product of hæmoglobin.

Clinical Uses of Vitamin E

The clinical uses of vitamin E are not well defined, and this is understandable when the confused picture of its mode of action and its physiological rôle is considered. It was somewhat inadvisably described as an antisterility factor and thought to be necessary to promote conception in the female. This is certainly not so. Conception does take place in E-deficient animals, but the subsequent pregnancy is by no means uneventful, and probably will not go to term. In rats some or all of the fœtuses are resorbed and in the human female abortion is probable. Possibly fœtal resorption in rats and abortion in humans are more or less analogous phenomena.

In male animals it may be that vitamin E is an antisterility factor. It appears to be essential for chromatin formation and may have some effect on the viability and gross morphology of spermatozoa as well as on their formation by the germinal epithelium of the testes. Vitamin E is therefore recommended for use collaterally with a gonadotropic hormone in the treatment of male sterility in which there is an abnormal sperm picture.

Vitamin E is undoubtedly an essential factor in the reproductive processes in both sexes, but too much was expected of it, so that the results of its clinical application proved to be somewhat disappointing. The other functions of the vitamin, acting either directly or through the pituitary gland, were then investigated, particularly its action in muscle metabolism.

Again the results of its use in muscular dystrophies were not encouraging, neither when it was given alone nor collaterally with other drugs.

Except for Shute's recommendation of the use of vitamin E as an anticœstrogen in certain cases of vulvo-vaginitis which are unresponsive to or are aggravated by œstrogens, present knowledge does not generally justify the use of vitamin E alone. Collaterally with other forms of treatment it may be of considerable value.

Hair and Sym (*Brit. Med. Journ.* July 3, 1943, p. 8) reported upon the use of vitamin E for the control of menopausal flushing. Their cases were too few to permit of definite conclusions as to its value, but they consider that the indications were that it is worthy of more extended trial. They question Shute's hypothesis of an anticœstrogenic action for vitamin E and suggest that it acts through the pituitary. The presence of any marked degree of hyperthyroidism in a menopausal patient they consider is an indication that the patient should receive œstrogen therapy to control the flushes and to inhibit the hyperactive pituitary. The patients most likely to respond to vitamin E are those in whom the flushes accompany some degree of hypothyroidism.

Daily Requirement of Vitamin E

There is no reliable evidence indicating what the daily requirement of vitamin E is for humans, but a minimum of 3 mg. to 5 mg. is suggested.

Administration and Dosage of Vitamin E

Vitamin E is almost invariably administered orally. It is readily absorbed and not inactivated by the secretions of the alimentary tract. Some patients may find the vitamin slightly nauseating and apt to precipitate gastric hyperacidity, although the vitamin is commonly administered in gelatin capsules in solution in oil. An alkali such as magnesium hydroxide may be given collaterally to minimise these effects.

Unit and Potency of Vitamin E

An international unit of vitamin E has been defined, subject to confirmation by the United Nations Health

Organisation when this becomes possible. A provisional international standard preparation has been issued by the Institute for Medical Research, Hampstead, and this consists of synthetic *dl*-α-tocopherol, 1 milligram of which has an activity of 1 international unit.

The international unit has not come into general use and appears to be redundant. The activity of preparations containing vitamin E, even concentrates containing mixed tocopherols, is expressed in terms of their equivalence to a definite weight (in milligrams) of α-tocopherol.

A biological unit has been used to some extent in the past. This is the Pacini-Linn unit, equivalent to $\frac{1}{10}$ to $\frac{1}{2}$ mg. of α-tocopherol.

Condition	Dose of Vitamin E	Principal Treatment
Abortion, habitual	6 mg. daily throughout pregnancy	Luteinising gonadotropin
Abortion, threatened	12 mg. to 18 mg. daily until delivery	Progestin
Amyotrophic lateral sclerosis	30 mg. to 200 mg. daily	—
Menopausal flushing	12 mg. or more daily for three weeks, then 6 mg. daily for at least two weeks*	—
Muscular dystrophies	Not less than 30 mg. daily	Glycine, ephedrine, Prostigmine, guanidine hydrochloride
Post-diphtheric paralysis	6 mg. to 18 mg. daily	—
Male sterility	6 mg. to 18 mg. daily	Serum gonadotropin
Hæmorrhagic pregnancy toxæmia	6 mg. to 250 mg., rarely 500 mg.†	*Note.*—Shute recommends fresh wheat-germ oil, but the equivalent doses in terms of the pure vitamin are given.

* Hain and Sym, *Brit. Med. Journ.*, July 3, 1943, p. 8.
† Shute, Vitamin E, A Symposium. *Soc. of Chem. Indus.*, 1939.

Doses of vitamin E are still defined or prescribed in terms of volume of wheat-germ oil. In addition to the rapid inactivation of vitamin E in wheat-germ oil, oils are very variable

in their original content of the vitamin, so that this method of specifying doses is highly inaccurate.

An average content of α-tocopherol of wheat-germ oil from figures given by Karrer and Keller, Emmerie and Engel, and by Furter and Meyer is 0·2 per cent.—that is, 2 mg. per c.c. Davison (*Synopsis of Materia Medica, Toxicology and Pharmacology*, 1944) gives 3 mg. in 4 c.c., and 500 mg. of wheat germ oil has also been stated to be equivalent to 3 mg. of α-tocopherol.

CHAPTER XIX

VITAMIN K

THE discovery of vitamin K in 1935 was the outcome of the apparently unpractical and academic work of Dam in Copenhagen. The speed with which the vitamin was recognised, isolated and identified was particularly remarkable; so also was the speed of the recognition of its significance in human physiology and its application in clinical medicine.

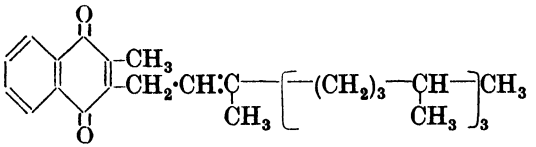
The vitamin was reported upon by Dam and Schonheyder (*Nature*, 1935, **135**, 652). Dam had observed, during investigations into steroid metabolism, a hæmorrhagic tendency in newly hatched chicks which he found could be prevented by the administration of a steroid-free, unsaponifiable fraction obtainable from hog's liver or alfalfa. He named the active substance vitamin K, from " koagulations vitamin " of the Scandinavian languages.

Vitamin K from alfalfa was eventually shown to be a phytyl derivative of naphthaquinone, and a second vitamin K, probably a difarnesyl derivative, was prepared from decaying fish meal. These two vitamins then became known as vitamin K_1 and vitamin K_2 respectively. The part of the molecule essential for the manifestation of vitamin-K activity is the naphthaquinone nucleus, and a number of other naturally occurring related substances have been found to possess vitamin-K activity, phthiocol, for example, prepared from the pigment of the tubercle bacillus, *mycobacterium tuberculosis*.

The next step was the preparation of various synthetic analogues of the natural vitamins. One of these, menaphthone (known as menadione in the U.S.A.), has become the standard " vitamin K ". The numbering of the series was continued for menaphthone (known occasionally as vitamin K_3) and for 2-methyl-4-amino-naphthol hydrochloride, vitamin K_5. Beyond this point the system is usually abandoned, and much confusion is avoided by the use of chemical names or short descriptive names.

Chemistry of Vitamin K

All the " vitamins K ", natural and synthetic, so far described are derivatives of naphthaquinone or naphthahydroquinone. Vitamins K_1 and K_2 were synthesised in 1939, and K_1 has the following structure :—



that is, 2-methyl-3-phytyl-1 : 4-naphthaquinone, K_2 being 2-methyl-3-difarnesyl-1 : 4-naphthaquinone. Among the synthetic analogues of the two natural vitamins, many radicles appear in positions 2 and 3, the 1 and 4 positions being occupied by either O or OH.

Menaphthone, the official " vitamin K " for administration by intramuscular injection in 2-methyl-1 : 4-naphthaquinone and its diacetyl derivative, acetomenaphthone (1 : 4-diacetoxy-2-methylnaphthalene) is the official substance for oral administration. There are many other proprietary substances, some of which are water soluble.

Generally, a methyl group on the quinone ring increases the vitamin K activity, and a hydroxy group diminishes the activity. Menaphthone, 2-methyl-1 : 4-naphthaquinone, for example, is highly active, and phthiocol, 2-methyl-3-hydroxy-1 : 4-naphthaquinone, is less active.

The " vitamins K " are readily inactivated by light, but are otherwise reasonably stable. The natural forms and many of the synthetic ones are soluble in oil, but a number of active synthetic substances have been prepared which are soluble in water.

Units and Potency of Vitamin K

There is no international unit for vitamin K. Menaphthone is generally regarded as the standard preparation, and doses of this are given in milligrams. A large number of biological or biochemical units have been used to some extent in the past, and these units are still found occasionally in the literature. Further, doses of proprietary preparations are sometimes given

in one or other of these units. For this reason it may be desirable to give the equivalents of the more important of these units (approximately) in terms of menaphthone, 1 mg. of which is equivalent to about :—

25,000 Dam units
700 Almqvist units
2,000 Ansbacher units
1,700 Thayer-Doisy units

Physiological Action of Vitamin K

Vitamin K is not stored in the body to any appreciable extent, but there are normally two sources of intake. Vitamin K is found in many vegetables, principally green leaves such as cabbage or spinach. This source is inadequate for normal needs, and the bulk of the daily requirement is probably absorbed as vitamin K_2 from the intestine, where it is formed by bacterial action on the intestinal contents.

The mode of action of vitamin K is unknown, but its principal and perhaps sole function is to promote the formation of prothrombin. It has been suggested that vitamin K, or at least the essential part of its molecule, enters into and becomes an essential part of the prothrombin molecule. Definite evidence of this does not appear to have been produced, and it is more likely that the vitamin plays an essential part in the formation of prothrombin, but does not itself enter into its structure. Another suggestion is that the vitamin promotes prothrombin formation merely as a result of stimulating the liver, the probable site of formation of prothrombin. The structure of prothrombin is unknown, but it has been suggested that the molecule consists of two parts, A and B (presumably proteins or polypeptides), linked together through a calcium atom (*Journ. Amer. Med. Assoc.*, March 11, 1944, 9, 734). Perhaps vitamin K plays a part in uniting the protein groups to the calcium.

A medicinal use for vitamin K has been suggested which is perhaps not connected with its vitamin activity. It has been shown that 1 mg. of menaphthone in 100 c.c. of saliva inhibits the formation of acid from 10 per cent. glucose, *in vitro* (*Science*, 1942, 96, 45). This finding has led to the suggestion that

menaphthone should be incorporated in sweets in order to prevent dental decay.

Clinical Uses of Vitamin K

Briefly, vitamin K is indicated for the prevention and treatment of those hæmorrhagic states which are known to be due to a lack of prothrombin (hypoprothrombinæmia).

Vitamin K should be administered as a routine to all pregnant women shortly before the onset of labour as a means of preventing intracranial hæmorrhage in the infant as a result of birth trauma.

A serious degree of intracranial hæmorrhage in newly born infants is not especially common, but the effect of even a comparatively slight deficiency on the mentality of infants can be so serious that it is advisable to take such a simple precaution as giving two or three doses of the vitamin to the mother just before labour begins. Further, slight intracranial hæmorrhage is possibly much more common than is generally supposed, and this may have undesirable effects which normally pass unnoticed. Although hyperprothrombinæmia appears to be usual during pregnancy (*Nature*, Feb. 24, 1940, p. 305), the administration of vitamin K as a routine is not likely to produce any undesirable effects in the mother (*Journ. Amer. Med. Assoc.*, Dec. 16, 1939, p. 2223), and it may well prove to be a factor in limiting post-partum hæmorrhage.

Hypoprothrominæmia is especially common in parous women, and the infants of such women, especially if barbiturate analgesics have been given during labour and if liquid paraffin has been taken regularly during pregnancy (*ibid.*, Nov. 22, 1941, p. 118). Gold compounds and arsenicals as well as barbiturates also cause hypoprothrominæmia, and it is therefore important to give vitamin K to all women just before labour is due to begin if they have received any of these drugs. Some degree of hypoprothrominæmia is normal in infants during the period when the prothrobin supplied from the maternal blood begins to diminish at about the third day until the establishment of intestinal bacteria permits of the production of vitamin K, and so enables the infant to form its own prothrombin. During this period there is a risk of serious hæmorrhage following apparently trifling injury, especially in premature infants.

Because of the production of vitamin K by intestinal organisms in normal persons, a purely nutritional hypovitaminosis-K is practically impossible, but deficiencies do frequently occur, either through non-absorption of the vitamin or through failure of production. The use of efficient intestinal antiseptics or of sulphonamides which act mainly in the intestine (such as sulphaguanidine, succinylsulphathiazole or phthalylsulphathiazole) may also cause a vitamin-K deficiency by inactivating the bacteria which produce vitamin K as well as other vitamins, notably those of the vitamin-B group.

Biliary deficiencies from hepatic malfunction, or from interference or interruption of bile flow, result in non-absorption of fats and fat-soluble vitamins, including K. The resultant hypoprothrombinæmia constitutes a serious danger in patients who are to undergo operations, as for obstructive jaundice, and the vitamin should be given in order to eliminate the hæmorrhagic tendency before surgical procedures are undertaken.

Vitamin K is specific in promoting prothrombin formation in all such cases as have so far been described, but it cannot be expected to produce any beneficial effects if there is marked impairment of the functions of the liver (including prothrombin formation), as in acute hepatitis, alcoholic cirrhosis, secondary hepatic carcinoma or acute yellow atrophy.

Vitamin K is of no value in hæmophilia, for in this condition the deficiency appears to be one of thrombokinase (thromboplastin) and not of prothrombin.

Excessive menstrual hæmorrhage may be due to a variety of causes. Hypoprothrombinæmia may be one of these, but more probably it is a contributory factor rather than a primary cause in a considerable proportion of the cases. Thus vitamin K may be valuable for auxiliary treatment for some patients, but it would perhaps be unwise to rely upon it to produce complete correction of the condition.

Similarly, vitamin K should not be relied upon to correct the diminished coagulability of blood following the administration of the natural anticoagulant heparin, or the synthetic dicoumarin. The action of both these anticoagulants is most effectively inhibited by blood transfusion or the infusion of whole blood, since by this means thrombokinase and thrombin are supplied.

Thus vitamin K is indicated for the prevention and control of hæmorrhage in the puerperium, intracranial hæmorrhage in the newly-born, and post-operative hæmorrhage in obstructive jaundice.

Intestinal diseases such as regional ileitis, ulcerative colitis, polyposis coli, idiopathic steatorrhœa and intestinal neoplasm interfere with the absorption of vitamin K, and administration of the vitamin is necessary to overcome the hypoprothrombinæmia and consequent hæmorrhagic tendency.

When the prothrombin has decreased to about 35 per cent. of its normal level, hæmorrhage occurs at the site of obvious trauma. Below 35 per cent. the hæmorrhage is provoked by minor trauma and hæmatoma may form from bruises and unnoticed minor injuries. The prothrombin level may fall to 15 per cent. to 20 per cent. of normal in newly born infants, in patients with idiopathic steatorrhœa, obstructive jaundice or parenchymatous disease of the liver. Spontaneous hæmorrhagic diathesis then becomes apparent, and may take the form of hæmarthrosis, hæmatemesis, epistaxis, hæmaturia, melæna, menorrhagia and intracranial and retinal hæmorrhage.

Prothrombin is readily destroyed by heat (40° C.–104° F.) and prolonged fever or artificial fever therapy may precipitate a hæmorrhagic tendency.

Daily Requirement of Vitamin K

Since the bulk of the normal daily intake of vitamin K is probably provided by the intestinal bacteria, the daily requirement is unknown, although Dam has suggested 50,000 Dam units or 1 to 2½ mg. of menaphthone.

Dosage and Administration of Vitamin K

The dosage of vitamin K generally recommended in the U.S.A. tends to be somewhat lower than that employed in Great Britain. The Council on Pharmacy and Chemistry of the American Medical Association has suggested 1 to 2 mg. of menadione (menaphthone) daily (*Journ. Amer. Med. Assoc.*, Jan. 17, 1942, p. 226), and added that "The dose should not exceed 2 mg. a day and should be continued at 2 mg. a day for a period exceeding four weeks."

The British Pharmacopœia (Sixth Addendum) gives a dose

of 5 mg. to 10 mg. by intramuscular injection without specifying the frequency of dosage or the duration of treatment. The dose of acetmenaphthone is given as 10 mg. to 60 mg., which is of course given orally.

The following are the principal conditions in which vitamin K is indicated and the doses which are suitable for general use in clinical practice. Doses by intramuscular injection refer to menaphthone and by the oral route to acetomenaphthone.

Obstructive Jaundice—5 mg. to 10 mg. intramuscularly, daily for two or three days before operation and 5 mg. daily for two or three days after operation.

Sprue and cœliac disease—10 mg. intramuscularly daily for five days and 10 mg. at intervals of two or three weeks.

Hepatic cirrhosis, or atrophy or chronic hepatitis—5 mg. daily or on alternate days intramuscularly.

During Pregnancy—Doses given vary considerably and either the intramuscular or oral route may be employed—5 mg. intramuscularly daily three or four days before labour is expected to begin. Alternatively, 50 mg. by mouth, in two doses (one of 30 mg. and one of 20 mg.) given within an hour of each other and the two within twenty-four hours of the onset of labour.

Neonatal hæmorrhage—10 mg. orally immediately before a feed and within twenty-four hours of birth. If there is evidence of hæmorrhage and a rapid effect is essential 5 mg. may be given intramuscularly. This involves some risk of local reaction in infants, and the oral route should therefore be employed whenever possible.

As has already been stated, vitamin K is best given to the mother just before labour begins for the prevention of hæmorrhage in newly born infants.

Although the method has apparently not been adopted, it has been suggested that vitamin K should be given as a routine by the percutaneous route to newly born infants (*Journ. Amer. Med. Assoc.*, Dec. 5, 1942, p. 1162).

When given to control menorrhagia or metrorrhagia, doses of 10 mg. intramuscularly together with 10 mg. orally have been suggested, daily, for five days (*Münch. Med. Wschr.*,

Sept. 12, 1941, per *Journ. Amer. Med. Assoc.*, July 25, 1942, p. 1060).

Hypervitaminosis-K

Unlike the other fat-soluble vitamins, vitamin K is not stored in the body to any appreciable extent by reason of its ready inactivation. Hypervitaminosis is therefore not produced following administration of therapeutic doses over long periods. Further, the administration of large single doses has not been observed to produce adverse effects. It is advisable, therefore, to give generous doses of vitamin K in order to ensure adequate therapeutic response. Untoward reactions are not to be anticipated if this is done.

Supplementary Note on Vitamin T

The existence of vitamin T is doubtful, but there is some evidence which seems to merit consideration and to warrant further investigation. About 1931 claims were made that vitamin A was concerned in blood-clotting, but this was subsequently disproved as purer preparations became available. It is possible that there is an impurity in the cruder preparations of vitamin A which may be identical with Schiff and Hirschberger's postulated factor T (1936). Factor T was stated to be present in egg yolk and in sesame oil. It seems to increase the platelet count in rat blood and in human blood, and the effect was said to be produced by doses of five to ten drops of sesame oil or by five egg yolks. Other investigators have suggested that doses of 1 oz. of sesame oil are necessary.

A suggestion that vitamin T may occur in association with vitamin A was made in the *Lancet* of February 21, 1942, p. 248.

Should the existence of vitamin T be substantiated and its function of raising platelet count be confirmed, the vitamin must take its place with vitamin K as an essential factor in normal blood coagulation.

CHAPTER XX

WATER-SOLUBLE VITAMINS—VITAMIN B_1

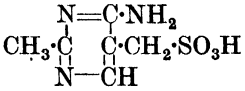
THE development of avian polyneuritis and of human beriberi in subjects subsisting mainly on a diet of polished rice was noted by Eijkman in 1897. Grijns, Eijkman's successor, established that polished rice did not contain a toxin, as Eijkman had suggested, but that it lacked an essential substance (1901).

Ten years later, Casimir Funk, a Polish biochemist, produced a crystalline substance from rice polishings which cured polyneuritis in pigeons in doses of 20 milligrams. For this preparation he introduced, in 1912, the name " vitamine," suggesting that it was an amine essential for life. Thereafter the name vitamine came into general use until in 1921 the final " e " was dropped in accordance with the suggestion of Drummond, who pointed out that all vitamins are not amines. The dual nature of " vitamine B " was realised before 1926, and it was quickly differentiated into the heat-labile and the heat-stable factors, the former being vitamin B_1 (vitamin B in the U.S.A.) and the latter vitamin B_2 (vitamin G in the U.S.A.). Vitamin B_2 was later to be subdivided into a considerable number of factors, but this will be considered in the chapter on vitamin B_2 (riboflavine). The principal factors of the vitamin B group, about which most is known, are aneurine, riboflavine and nicotinamide.

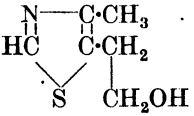
Attempts to purify Funk's original " vitamine B " began in 1912, and this was continued and other attempts to isolate the anti-beriberi factor were continued in the face of considerable difficulties, until in 1934 Williams *et al.* succeeded in obtaining 5 grammes of the pure hydrochloride from a ton of rice polishings (about 25 per cent. of its actual content).

Then began the elucidation of the molecular structure of the vitamin. Windaus, Tschesche and Grewe identified two substances produced from the vitamin by oxidation with nitric acid, $C_7H_{11}N_3O_5$ and $C_5H_5NO_2S$. Williams *et al.*, in 1935,

found that treatment with sodium sulphate in faintly acid solution at room temperature gave two compounds, a sulphonic acid compound :—



and a basic compound :—



The basic compound, on oxidation with nitric acid, gave a substance identical with the $C_5H_5NO_2S$ produced by Windaus *et al.* The sulphonic acid compound was shown to be a substituted 6-aminopyrimidine.

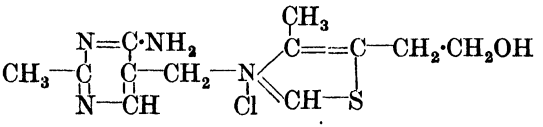
Various investigations confirmed a structure suggested by Williams, and the vitamin was synthesised by him in 1936 and later by several groups by various methods and shown be to identical with the natural vitamin.

Associated with vitamin B_1 in the thermolabile fraction of the vitamin B group is vitamin H, recognised in 1931 and identified with biotin in 1940 (see p. 256). The fraction is completed by the chemically unidentified vitamins B_3 and B_4. Reference to the separation and identification of the constituents of the thermostable vitamin B_2 group is made in the next chapter, on riboflavine (vitamin B_2).

Chemistry and Nomenclature of Vitamin B_1

The synonyms by which vitamin B_1 has been known are numerous. Some, now practically obsolete, indicate sources from which it has been obtained—torulin (torula = yeast) and oryzanin (oryza = rice). A third synonym indicates both its source and one of its physiological effects—catatorulin. The official American name, thiamin (or thiamine), is based on its chemical structure, and the British Pharmacopœial name indicates vaguely its function, aneurine, the antineuritic vitamin.

The chemical structure of vitamin B_1 was determined and the vitamin was synthesised in 1936. The structure is indicated by the following formula :—

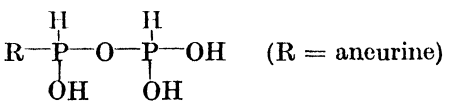


The systematic name describing this structure is 3-(4′-amino-2′-methylpyrimidyl-5′-methyl)-4-methyl-5-β-hydroxyethylthiazolium chloride. The official preparation of this, the vitamin itself, is the hydrochloride. The names aneurine and thiamine apply to the vitamin itself. The official preparation is correctly described, either as aneurine hydrochloride or thiamine hydrochloride, but the synonyms aneurine chloride hydrochloride and thiamine chloride hydrochloride sometimes used are thus inaccurate, and should be abandoned.

Aneurine is stable to heat except in neutral or alkaline solution, unlike the other members of the vitamin B group. In acid solution (pH 3) it is stable to heat, but in alkaline solution the molecule is readily ruptured at the carbon link between the pyrimidine and thiazole nuclei.

Oxidation of aneurine with alkaline potassium ferricyanide yields thiochrome, a pale-yellow substance with a blue fluorescence. This reaction forms the basis of a "fluorimetric" method of estimation of the vitamin.

Aneurine itself is inactive as a vitamin in the animal body until it has been phosphorylated to form the pyrophosphate,



Aneurine pyrophosphate is cocarboxylase, the coenzyme of carboxylase. (See Supplementary Note at end of chapter.) The enzyme itself, carboxylase, consists of aneurine pyrophosphate as a prosthetic group attached to a specific carrier protein through the hydroxyl groups of the pyrophosphate radicle. Magnesium is also necessary as an activator, and may either form part of the protein carrier molecule or simply be present in an ionised state. It is probable, however, that the complete enzyme is a diphosphoaneurine metalloprotein containing about 0·46 per cent. of diphosphoaneurine and 0·13 per cent. of magnesium. Manganese also functions as an activator of carboxylase, and appears to be considerably more

efficient, weight for weight. Any other divalent metal can replace magnesium, though usually they are less efficient.

Mode of Action of Vitamin B_1

Vitamin B_1 is the essential component (prosthetic group) in the carboxylase enzyme system which catalyses the decarboxylation of α-ketomonocarboxylic acids, α-ketovaleric and α-ketobutyric acids, but principally pyruvic acid.

Cocarboxylase (aneurine pyrophosphate) appears to be readily hydrolysed unless there is a sufficient amount of free aneurine present. The free vitamin has the property of inhibiting this hydrolysis.

The precise mode of action of the enzyme in bringing about decarboxylation is as yet unknown. Reduced to its simplest terms, the action of the carboxylase enzyme system is to catalyse the reaction :—

$$\underset{\text{(Pyruvic acid)}}{2CH_3{\cdot}CO{\cdot}COOH} + 5O_2 = 6CO_2 + 4H_2O$$

This is by no means the whole story, however, for other α-ketomonocarboxylic acids are similarly decarboxylated under its influence. Further, combination of the aneurine pyrophosphate radicle with other proteins produces specific carboxylases for the decarboxylation of other types of acids, and the enzyme is also an activator of certain phases of the so-called citric acid and succinic acid cycles which are linked up with pyruvic acid metabolism. These activities of the enzyme have not been fully elucidated, and any further discussion would be out of place at this point.

The phosphorylation of vitamin B_1 can be effected by many body-tissues, but most of the vitamin appears to be phosphorylated by the leucocytes. It is suggested that the reaction involved is :—

$$\text{Adenosinetriphosphate} + \text{vitamin } B_1 \longrightarrow \text{adenylic acid} + \text{cocarboxylase}$$

Adenosinetriphosphate appears to be an almost universal phosphate donor for metabolic and enzymatic processes, and this important substance is further considered on pages 211 and 254.

Most of the symptoms of vitamin B_1 deficiency (particularly

the neurological lesions) are probably the result of impaired ability to utilise glucose and its metabolic products properly. A high proportion of fats in a diet has a sparing action on vitamin B_1, for the fats can provide an alternative source of energy to glucose and the oxidation of the fats does not require the presence of vitamin B_1 or its derivatives. This vitamin-sparing action is most marked when the fats contain a high proportion of fatty acids containing eight carbon atom chains—for example, caprylic acid. The presence of choline seems to be essential, however, if fats are to replace glucose successfully as a source of energy.

Vitamin B_1 seems to have some direct but as yet unelucidated action on the thyroid gland. In deficiency states the gland undergoes colloid hypertrophy, and there is a decrease in active material. The anterior pituitary also undergoes some degeneration, and this probably contributes to the thyroid degeneration. Conversely, it has been stated that vitamin B_1 antagonises thyroxine and that if the vitamin and hormone are administered collaterally in suitable doses the toxic effects of the hormone are counteracted by the vitamin.

Further, vitamin B_1 potentiates the action of acetylcholine. Two mechanisms seem to be involved in this : first, the vitamin inhibits the action of choline esterase, and, second, in the presence of pyruvic acid and a high concentration of potassium ions, vitamin B_1 brings about the synthesis of acetylcholine.

Unit and Potency of Vitamin B_1

The international unit of vitamin B_1 was originally the antineuritic activity of 10 mg. of an adsorbate of the vitamin on fuller's earth. This has since been replaced (1938) with an international standard preparation of the hydrochloride of the pure vitamin. The international unit is essentially the same as the original unit, but it has been re-defined as the antineuritic activity of 3·33 γ (approx. $\frac{3}{1000}$ mg.) of the international standard preparation.

The aneurine hydrochloride of the B.P. is a hydrated compound and has an activity somewhat less than that of the international standard preparation (320 as compared with 333 international units per milligram).

Different test methods give various equivalents for activity

of weight of substance in terms of units, so that in the earlier literature various figures of 200 to 500 international units per milligram of standard preparation may be found. It is now agreed, however, that 1 mg. of the standard is equivalent to 333 units of the anhydrous, or 320 units of the hydrated, preparation.

Physiological Action of Vitamin B_1, and Deficiency Symptoms

The normal metabolic processes in which vitamin B_1 (as the active group in enzyme systems) plays an essential rôle are common to most body-cells, particularly those of nerve and muscle tissues. Symptoms of deficiency are correspondingly diverse and affect almost all body tissues in varying degree.

Platt and Lu (*Quart. Journ. Med.*, 1936, **5**, 355), have given the principal deficiency symptoms in relation to progressive degrees of deficiency. Four groups are given, and these include the clinical signs of hypovitaminosis in order of their appearance as the deficiency increases.

1. Neuritis, usually of legs, together with altered or absent knee and ankle jerks
 Paræsthesiæ
 Muscle weakness
2. Œdema, cardiac enlargement, tachycardia
3. Cyanosis, dyspnœa, cardiac decompensation
4. Epigastric distress, prostration, restlessness

Any one or a combination of several of these symptoms may appear in a case of clinical hypovitaminosis, and if most of them are present at any one time, a diagnosis of beriberi is justifiable.

In "dry" beriberi, œdema is absent, and the principal symptoms are neurological (neuritic or paraplegic beriberi). "Wet" beriberi is characterised by œdema, and this is accompanied by cardiovascular and respiratory symptoms—dyspnœa, palpitation and tachycardia.

Usually, however, the full beriberi syndrome is not present, only a few of the deficiency symptoms being present at any one time.

Uncomplicated deficiencies of any one member of the vitamin-B group do not occur except under strictly controlled

laboratory conditions. The dietary sources of the vitamins of the B group all contain nearly every member of the group, so that deficiency states of dietary origin are characterised by a syndrome of mixed symptoms. In many cases it is not possible to detect a predominating deficiency of any one member of the group. Diets which are unbalanced as far as bulk constituents are concerned, as distinct from being grossly deficient in vitamins, are more likely to produce a deficiency state in which symptoms of lack of one particular vitamin are mainly evident. For example, a high carbohydrate diet increases the need for vitamin B_1 in particular, and if this is not provided the vitamin-B_1 deficiency symptoms will appear more rapidly and will assume the dominant rôle in the syndrome. Thus a diet which contains little but polished rice does not supply the vitamin B group, it makes special demands on the little vitamin B_1 available and soon produces the full syndrome of beriberi. Vitamin B_1 is not stored in the body to any appreciable extent, but the body clings tenaciously to its supply of riboflavine. Nicotinamide is not depleted so readily as vitamin B_1, so that beriberi is predominantly a vitamin-B_1-deficiency condition, symptoms of deficiency of the other important members of the group appearing only in the more advanced stages of the condition.

Much the same applies to alcoholism, in which the predominating vitamin B_1 deficiency is manifested mainly by polyneuritis and anorexia. The anorexia is responsible for a diminished intake of the whole vitamin-B group, so that the condition not only tends to become chronic, but more and more complicating deficiency symptoms gradually appear as the disease progresses. The administration of vitamin B_1 only eliminates the major symptoms, but by restoring the appetite, tends to provide for an increased intake of the whole vitamin B group.

Polyneuritis also appears as a dominant symptom in pregnancy.

In this case the unusual metabolic state is such that more than normal amounts of vitamin B_1 are required. Nausea, vomiting and dietary idiosyncrasies reduce the intake of the vitamin, and the polyneuritis tends to increase, and other symptoms appear.

Thus, in the general course of clinical practice, deficiencies of vitamin B_1 are in the first instance usually conditioned deficiencies. That is, deranged metabolism or an abnormal diet increases the vitamin-B_1 requirement, and then this gives rise to a diminished intake and a consequent primary deficiency. A vicious circle is thus set up, and the condition of the patient tends to get steadily worse until the principal deficiency is corrected by the administration of the chief deficient substance, vitamin B_1. Thereafter the appetite improves, and if a reasonably balanced diet is taken, the associated deficiencies are corrected without specific medicinal treatment.

The clinical manifestations of vitamin B_1 deficiency are not only polyneuritis and anorexia. Indeed, should these symptoms only appear, it is probable that the patient is not suffering from a vitamin deficiency, and some other cause should be suspected. The premonitory symptoms are generally paræsthesiæ and tenderness of muscle tissues. Thereafter anorexia and polyneuritis may be expected to appear, and these may be accompanied by œdema, particularly of the ankles and calves, and by intestinal atony.

Confirmation of the diagnosis is possible by demonstrating the presence of increased amounts of " bisulphite binding substances " in the blood (chiefly pyruvic acid) and the virtual absence of vitamin B_1 from blood and urine. This is not an easy matter for the average general practitioner, however, and the most satisfactory procedure is probably the administration of the vitamin on symptomatic clinical evidence only. If the diagnosis is correct, a rapid response follows the administration of the vitamin in adequate doses.

Clinical Uses of Vitamin B_1

Prophylactic Uses.—The principal dietary sources of vitamin B_1 are lean meat, liver, nuts and whole or " high-extraction " wheat in the form of " breakfast cereals," flour and bread. No one of these items of diet can supply the full daily requirement, and the requirement is increased by raised metabolism and increased physical, mental and emotional activity. Thus there is a comparatively high risk of varying degrees of deficiency, especially if the carbohydrate intake is high and in alcoholics.

The physician, from a consideration of the patient's diet, conditions and symptoms, may thus arrive at a reasonably correct diagnosis of aneurine deficiency before definite symptoms of a clinical state of hypovitaminosis develop. Vitamin B_1 can then be prescribed prophylactically to prevent the development of serious deficiency states and to relieve deficiencies which impair efficiency and cause various minor and vague neuritic pains and impair metabolic efficiency. This is particularly the case during pregnancy, when the administration of vitamin B_1 may do much to relieve the minor derangements which are so often accepted by most women with resignation as more or less inevitable. Among these derangements are nausea, œdema, mild neuritis, anacidity and anorexia.

Therapeutic Uses.—The vitamin is of course indicated for the treatment of the classical deficiency symptoms which have been named in the previous section. Other conditions in which it has been reported to be of value are peripheral vascular disease, irradiation sickness, hyperemesis gravidarum and delirium tremens, especially that resulting from alcoholism and possibly associated with polyneuritis. If given in adequate doses, vitamin B_1 will give complete relief from the effects of alcoholism, even although the amount of alcohol taken is not reduced. In such cases the vitamin assists materially in restoring the appetite, and thus contributes indirectly as well as directly to the cure of the patient.

An uncomplicated deficiency of any one vitamin of the B group must be extremely rare, if it exists at all, apart from artificially induced deficiency produced by means of an elaborately prepared diet for the specific purpose of investigation. Nevertheless, deficiency of one vitamin commonly predominates in a multiple deficiency, and the administration of the pure vitamin is thus reasonable and justifiable. At the same time, in order to effect a complete cure, the patient should be given an improved diet in order to make good the associated secondary deficiencies. In some instances this may be inadequate, and it will then be necessary to administer one or more of the other members of the vitamin-B group or a concentrate containing most or all of them.

During pregnancy the vitamin-B requirements are specially high, and minor symptoms of deficiency are common. These

have already been mentioned in the section on prophylaxis. Such conditions as were mentioned may become sufficiently severe to become incapacitating, and larger doses of the vitamin are necessary to give relief. Severe neuritis or polyneuritis, marked paræsthesiæ and œdema, particularly of the legs, are outstanding symptoms of this type. Dietary idiosyncrasies are not uncommon among pregnant women, and these may result in an excessive intake of carbohydrates. Extra vitamin B_1 is necessary to deal with this.

Daily Requirement of Vitamin B_1

The normal average daily requirement of vitamin B_1 is of the order of 1 mg., but, as has already been indicated, a number of factors may increase this. For this reason it is being more and more realised that the formula suggested by Cowgill for calculating the requirement of an individual is not altogether reliable. The formula suggested was as follows :—

Vitamin B_1 requirement in international units
$= 0{\cdot}00142 \times$ weight in kilos $\times$ Calorie intake

This may be approximately correct for a *normal* person, but so-called normality is probably rather rare.

Another complicating factor is the synthesis of vitamins, especially those of the B group, in the normal intestine by bacteria. The amount of this is unknown, but it is probably considerably more than was thought to be so formed until quite recently. The use of sulphonamides, particularly in enteric disease, has brought this subject into prominence. The sulphonamides used in dysentery, especially sulphaguanidine and succinylsulphathiazole, are not absorbed rapidly or extensively and exert a marked antibacterial effect in the intestine. Patients undergoing such treatment are therefore particularly susceptible to general vitamin deficiency, whereas a normal person with a normal intestinal flora may live for considerable periods on a low dietary vitamin intake without showing any marked symptoms of vitamin deficiency.

The increased use of sulphonamides may thus, in some measure, account for the tendency to give much larger therapeutic doses of vitamin B_1 than would appear to be necessary on the basis of the daily requirement. Further, it is probable that vitamin B_1 when given in large doses has a

pharmacodynamic effect quite distinct from its vitamin effect. Similarly, vitamin D almost certainly has a pharmacodynamic effect which is probably quite independent of its vitamin activity.

Provisionally, it may be assumed that the average minimum daily requirement of vitamin B_1 of a normal adult is 3 mg. (about 1,000 international units) from dietary sources, together with an unknown amount synthesised by the coliform bacteria of the intestines from which it is absorbed. The desirable intake is probably about 5 mg. to 6 mg.

Administration and Dosage of Vitamin B_1

The oral route is commonly employed for the administration of vitamin B_1 unless persistent vomiting or a suspected hypochlorhydria are likely to interfere with absorption, or if particularly large doses are required which it would be inconvenient to administer orally. Further, some patients may find that doses of more than 5 mg. or 6 mg. of vitamin B_1 may produce an unduly laxative effect when taken orally. Thus it is usual to give doses in excess of 6 mg. parenterally, intravenously in acute conditions when an immediate response is required and subcutaneously (or less frequently intramuscularly) when an early response is not so imperative. Vitamin B_1 has been injected into the spinal column, but this has now been abandoned because there is a high incidence of reactions, hypersensitive patients may react especially violently, and even in the absence of reactions there are no appreciable advantages over administration by the intravenous route.

For the prevention of the onset of deficiency symptoms vitamin B_1 is given orally in doses of 1 mg. to 3 mg. daily. Such doses should be given whenever increased metabolism, dietary inadequacies, pregnancy, lactation or any other special circumstances suggest that the procedure is desirable.

If glucose is being given intravenously or if profuse diuresis has been produced, vitamin B_1 should be given prophylactically in doses of 5 mg. to 10 mg. daily.

The pharmacopœial prophylactic dose (B.P., Third Addendum, 1941) is smaller than is usually given in practice, and in view of what is known of the daily requirements of the vitamin, there is good reason to prescribe doses considerably in excess of the official ones, both prophylactically and therapeutically.

The official doses are 0·3 mg. to 0·6 mg. (prophylactic) and 0·6 mg. to 1·8 mg. (therapeutic) daily, the maximum being about one-third of the suggested desirable daily requirement. Thus the maximum therapeutic dose may be sufficient to prevent the onset of deficiency symptoms, but it cannot be expected to correct any actual deficiency.

THERAPEUTIC DOSAGE OF VITAMIN B_1

Condition	Dose	Remarks
Acrodynia—see Pink disease	—	—
Alcoholism (see also Delirium tremens)	10 mg. to 100 mg. daily in doses of not more than 25 mg. each	Intramuscular injection until symptoms are diminished, then oral administration
Beriberi	20 mg. to 40 mg. daily until improvement is noted, then 5 mg. to 10 mg. daily and eventually every two or three days	The first few doses may be given intravenously, and then intramuscular injection should be employed. The smaller doses may be given orally, together with a diet rich in the vitamin B group
Delirium tremens	20 mg. to 80 mg. hourly for three doses intramuscularly	Thereafter treatment as for alcoholism
Hyperemesis gravidarum	20 mg. to 50 mg. daily intramuscularly	—
Neuritis	5 mg. to 50 mg. once or twice daily	Intramuscular injection until appreciable relief is obtained, then oral administration
Peripheral neuropathy in diabetics	10 mg. daily orally	—
Pink disease	2 mg. to 5 mg. daily or 5 mg. to 15 mg. once or twice weekly	The first few doses may be intramuscular, then oral. Vitamin E and pyridoxine have been given collaterally.
Post-diphtheric paralysis	2 mg. to 10 mg. daily intramuscularly	—

Hypervitaminosis-B_1 and Allergic Reactions to the Vitamin

There is very considerable variation in patients' toleration of large doses of vitamin B_1. Some experience a laxative effect

and excessive intestinal motility following the ingestion of doses of 3 mg. to 5 mg., while others, especially if they are deficient in the vitamin, tolerate doses of the order of 100 mg. to 500 mg., even by the intravenous route. Still other patients appear to develop allergic hypersensitivity to the vitamin administered medicinally. In monkeys 600 mg. or more per kilo produce toxic effects, manifested, as in most animals, by muscular weakness and tetany, spasm, slow and irregular breathing followed eventually by respiratory failure.

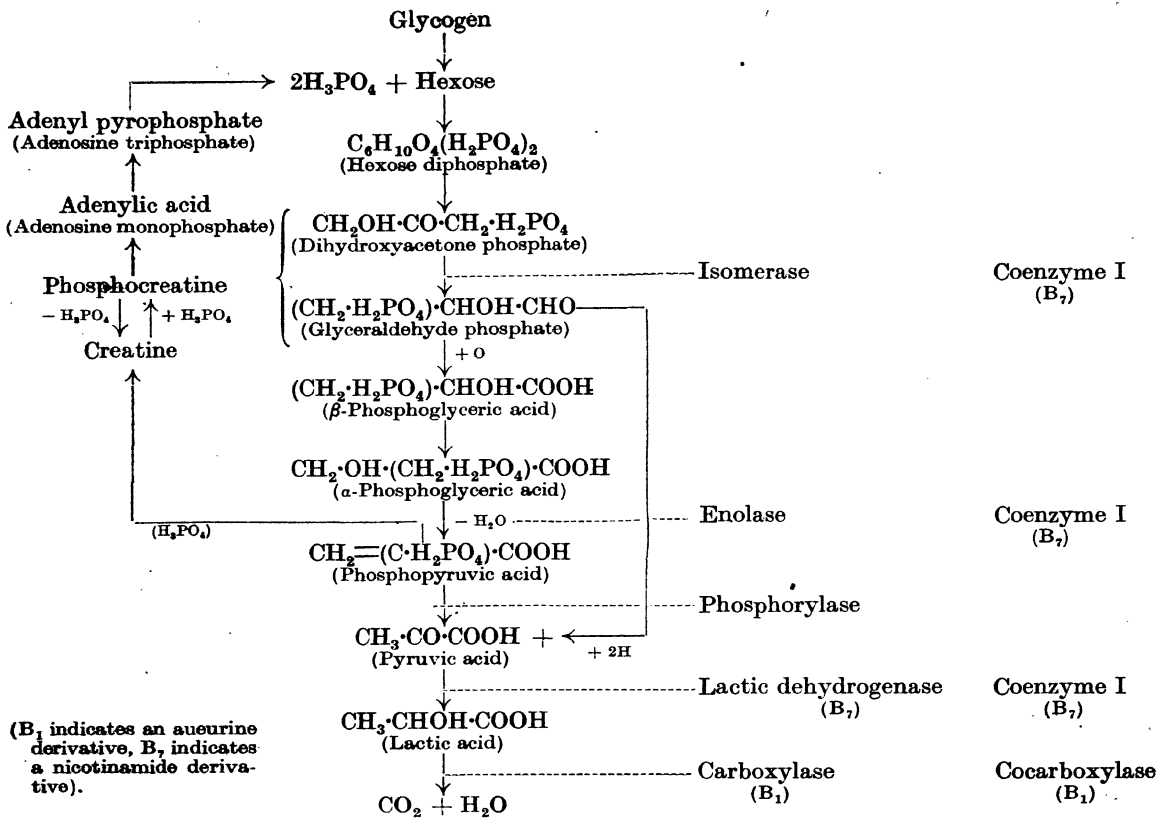
The enormous difference between therapeutic and toxic doses of vitamin B_1 makes hypervitaminosis in man practically impossible.

Little is known of the factors controlling the development of allergic hypersensitivity to vitamin B_1. The symptoms are those typical of allergic states, urticaria in mild cases, and cases have been reported of respiratory distress and collapse. Adrenaline gives immediate control and subsequently desensitisation can be carried out so that treatment with the vitamin can eventually be resumed.

Beneficial effects have been claimed following the intraspinal injection of vitamin B_1 in such conditions as Korsakoff's psychosis and dementia, but this method of administration is now rarely attempted, since it is realised that there is a considerable risk of producing severe reactions. These reactions may involve injury to tissues of the central nervous system similar to those produced in some cases of hypersensitivity to the vitamin—for example, subpial ecchymoses and encephalomalacia. These and other symptoms resembling those of hyperthyroidism are characteristic of an allergy to vitamin B_1 which has been reported upon in tropical regions, where vitamin B_1 requirements are high and intakes tend to be low. It is possible that some cases of hypersensitivity in temperate regions may show some of the symptoms seen in the tropics; these include nervousness, insomnia, headache, tremor, rapid pulse, hypertension, emotional instability, nausea and vomiting, as well as perivascular hæmorrhage.

Supplementary Note

As has already been stated, aneurine and nicotinamide are the active prosthetic groups of the enzymes which catalyse the oxidative breakdown of the carbohydrates. The


Glycogen
2H3PO4 + Hexose
Adenyl pyrophosphate
(Adenosine triphosphate)
C6H10O4(H2PO4)2
(Hexose diphosphate)
Adenylic acid
(Adenosine monophosphate)
CH2OH·CO·CH2·H2PO4
(Dihydroxyacetone phosphate)
Isomerase
Coenzyme I
(B7)
Phosphocreatine
– H3PO4
+ H3PO4
(CH2·H2PO4)·CHOH·CHO
(Glyceraldehyde phosphate)
Creatine
+ O
(CH2·H2PO4)·CHOH·COOH
(β-Phosphoglyceric acid)
CH2·OH·(CH2·H2PO4)·COOH
(α-Phosphoglyceric acid)
– H2O
Enolase
Coenzyme I
(B7)
(H3PO4)
CH2=(C·H2PO4)·COOH
(Phosphopyruvic acid)
Phosphorylase
CH3·CO·COOH +
(Pyruvic acid)
+ 2H
Lactic dehydrogenase
(B7)
Coenzyme I
(B7)
CH3·CHOH·COOH
(Lactic acid)
Carboxylase
(B1)
Cocarboxylase
(B1)
CO2 + H2O
(B1 indicates an aneurine derivative, B7 indicates a nicotinamide derivative).

process provides the energy necessary for muscular contraction. It is a complicated process, and all the details are by no means understood. A much-simplified scheme indicating the main outline of the process can be given (see previous page), and the points of action of the principal enzymes and coenzymes indicated.

In column 2 the carbohydrates and their oxidation products are named. Column 3 contains some of the principal enzymes catalysing certain of the reactions. Those enzymes which catalyse oxidations (dehydrogenases) require coenzyme I (cozymase) as recipients of hydrogen, and these in turn pass the enzyme on through intermediates (the cytochromes) to diaphorase (a flavoprotein enzyme, containing riboflavine), from which it is passed on to molecular oxygen (from the erythrocytes) to form water. The red blood cells thus bring oxygen for combination with the hydrogen of carbohydrate catabolism, and carry away carbon dioxide also produced in this catabolic process. The phosphoric acid involved is removed at the phosphopyruvic acid–pyruvic acid stage of the catalysis, and conveyed by the creatine–adenylic acid cycle back to phosphorylate more hexose (column 1). It seems probable that adenylpyrophosphate loses two molecules of phosphoric acid, not directly to the hexose, but via myosin, the globin of the muscle fibres. Myosin is thus in a sense a coenzyme or "phosphoric acid acceptor." The molecule of myosin appears to be a straight-chain compound, but when it is phosphorylated the chain becomes folded, and so shorter. The summation of this shortening of the molecules appears to constitute the contraction of muscle fibres and thus of the muscles. Repetition of this process results in an accumulation of pyruvic and lactic acids. Removal, or rather decomposition of these metabolites, is the function of lactic dehydrogenase (which has a reversible action and so can "dehydrogenate" as well as hydrogenate), and of carboxylase, together with their respective coenzymes (or "dehydrogenators"), the coenzymes passing this hydrogen on to diaphorase as already indicated.

The importance of these complex compounds of aneurine and nicotinamide will thus be readily apparent and the serious consequences of deficiencies realised.

CHAPTER XXI

VITAMIN B_2 (RIBOFLAVINE)

FOLLOWING the separation of vitamin " B " into the thermostable and thermolabile fractions in 1926, it was not until 1930 that any further subdivision of the former fraction was effected. In this year it began to be generally accepted that the thermostable fraction appeared to contain a substance essential for rat-growth and maintenance of weight in pigeons. The identity of this factor has still not been established (vitamin B_5), but it is suspected that it is identical with vitamin B_6 (pyridoxine).

In 1932 Warburg and Christian separated their " yellow enzyme " from yeast and split this into a protein-carrier and a pigment component. In the following year Ellinger and Koschara called attention to the wide distribution of yellow pigments with a green fluorescence in milk, liver, kidneys, avian, muscle, yeast etc., and suggested that these substances were identical and that the pigment was the same as the pigment component of Warburg and Christian's yellow enzyme. Kuhn *et al.* (1933) suggested that the suffix " flavine " should be used in naming these pigments, the identity of which with one another they were as yet apparently unable to accept. Hence arose the names hepatoflavine, ovoflavine and lactoflavine. Eventually the identity of these substances was established, and it was shown that the pentose sugar ribose was a constituent of the pigment. Following this the name riboflavine was accepted, and has largely replaced the name vitamin B_2.

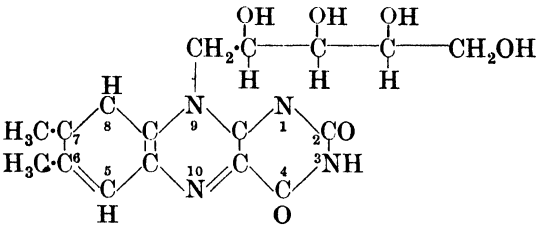
The isolation of riboflavine in 1934 was followed by the establishment of its chemical constitution on its synthesis in 1935 by Kuhn and by Karrer *et al.*

Chemistry of Vitamin B_2

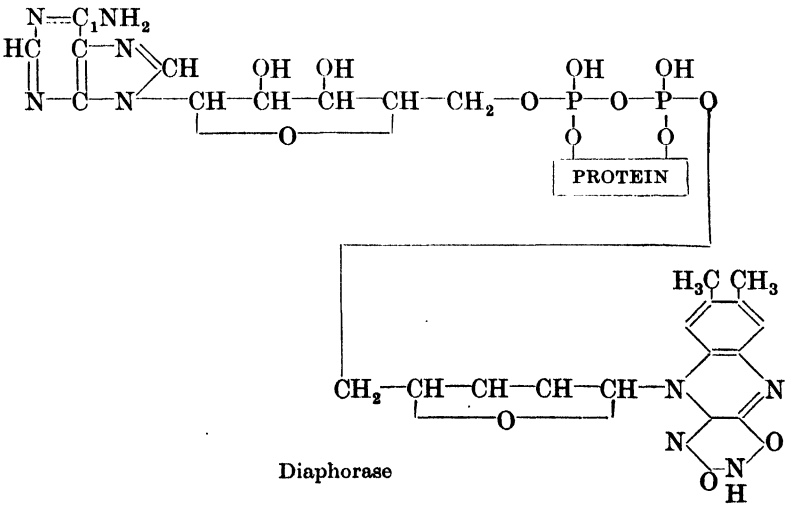
Vitamin B_2 (riboflavine) occurs in yellowish-brown acicular crystals which have no sharp melting point, but which decom-

pose between 267° and 294° C. It is quite insoluble in fat solvents and only soluble in 400 parts of water at 25° C. It is stable in acid solution, but decomposes in alkaline solution and when exposed to ultraviolet light.

The constitution of riboflavine is indicated by the name 6 : 7-dimethyl-9-*d*-ribityl-*iso*alloxazine and by the following structural formula :—



This forms a prosthetic group of a number of enzymes and coenzymes, probably at least seven. Among these are Warburg's yellow enzyme, flavine-adenine dinucleotide and the diaphorases. Warburg's yellow enzyme consists of riboflavine linked through the ribose radicle through a molecule of phosphoric acid to a protein carrier group. Flavine-adenine dinucleotide also contains riboflavine attached to two



Diaphorase

phosphoric acid groups, and through them and a second molecule of ribose to adenine. There are a number of diaphorases, and

each consists of flavine-adenine dinucleotide linked through the phosphoric acid radicles to a specific protein. The individuality of the diaphorases depends on the nature of the protein. The structure of diaphorase is given, and from this the structure of the yellow enzyme and flavine-adenine dinucleotide can be deduced.

All the riboflavine compounds are conveyors or acceptors of hydrogen. The nitrogen atom at position 10 in the isoalloxazine condensed ring system is the functional atom and acts as the actual point of attachment of the hydrogen.

The flavine enzymes together with the cytochromes form the intermediaries through which the hydrogen produced by the oxidative decomposition of carbohydrate is conveyed, through the dehydrogenases and their coenzymes, to be combined with the oxygen supplied by the red blood cells (see Supplementary Note to the previous chapter). The mechanism is complex, and has not yet been elucidated in all its details. The overall effect can be summarised as follows :—

$$\text{Substrate} + H_2O + E_2 \longrightarrow \text{oxidised substrate} + H_2O_2$$
(*e.g.*, lactic acid)

This takes place in stages, and the process can be represented in somewhat more detail by the following series of reactions :—

Substrate + dehydrogenase ⟶ activated substrate. Activated substrate + H_2O + coenzyme ⟶ reduced coenzyme + oxidised substrate. Reduced coenzyme + flavoprotein ⟶ coenzyme + reduced flavoprotein. Reduced flavoprotein + O_2 ⟶ regenerated flavoprotein + H_2O_2

Similar mechanisms exist for the oxidative decomposition of amino-acids, lactic acid and aldehydes.

It is in connection with the carbohydrates that riboflavine has been studied most intensively, but it appears that in normal circumstances the same functions are carried out by hæmin compounds. The riboflavine system of enzymes appears to be an alternative to the hæmins, which can replace them to some extent in abnormal circumstances or when the hæmins are deficient. Exceptions to this are the avascular tissues of the body, such as those of the eye, and perhaps some of the upper layers of mucous membranes which are not

efficiently served by the capillaries. It appears that the outstanding example of this is the mucocutaneous junction at the angles of the mouth.

Units and Potency of Vitamin B_2

The full importance and significance of vitamin B_2 have probably yet to be realised. Hitherto it has not been considered to be of sufficient importance to justify the formulation of an international unit, and now that it has been isolated, synthesised and thoroughly characterised it is not necessary to introduce international standards. The pure substance is made synthetically as a routine, and doses can be expressed in terms of weight.

Biological units have been defined, however, and these are still employed to some extent in the literature. For example, there is at least one rat unit, probably the growth-promoting effect of 4 micrograms. The unit in most common use, however, is the Bourquin–Sherman unit (also a rat-growth unit), which is generally accepted as being equivalent to 2·5 micrograms. Thus 1 mg. of riboflavine is equal to 400 Bourquin–Sherman units.

Physiological Action of Vitamin B_2

As has been indicated, the chemistry of the riboflavine compounds active in the body is known in some detail, but although the mechanism of their function in the oxidation–reduction systems is understood, details of the relationship of derangements caused by deficiencies to the clinical symptoms of deficiencies are practically unknown.

Stannus in his Lumleian Lectures, 1944, (*Brit. Med. Journ.*, July 22, p. 103, and July 29, p. 140), gives a comprehensive survey of the literature on the subject of riboflavine deficiency, and from this evidence, and from his personal observations, makes what is probably the most satisfactory and comprehensive suggestion as to how deficiencies cause the observed clinical symptoms. The original lectures as reproduced in the journals (see above) should be studied, but a brief summary may be given here.

First Stannus attempts to " disengage from the clinical affection we call pellagra a group of symptoms which may be met with as a more or less complete syndrome apart from

those symptoms generally recognised as due to a nicotinic acid deficiency ".

The American authors have described a syndrome which they have called aribоflavinosis. Stannus includes a wider range of symptoms and rightly prefers to call it *hypo*riboflavinosis, for, as he points out, death comes before complete deficiency can be produced, so that *a*riboflavinosis is never encountered.

Stannus points out that it is not yet possible to state categorically whether or not deficiency of any other factor in the vitamin B group is concerned in the syndrome he calls hyporiboflavinosis, but it is evident that lack of riboflavine is the principal cause of the symptoms. The fundamental effect of deficiency is " a reversible functional disturbance of the capillary endothelium—a ' capillary dysergia '—which at the present time cannot be more accurately defined, but which is manifested by dilatation of these vessels and impaired flow. . . . The interference with tissue-cell metabolism is probably of the nature of an anoxia (or hypoxia) in its wide sense.

" It is suggested that disorders of capillary function—' dysergia '—quite apart from affections of the rest of the vascular system, may play a much more important part than has commonly been supposed in the pathogenesis of many conditions, including pellagra due to nicotinic acid deficiency.

" At the risk of appearing mono-ideistic, I cannot help feeling there is perhaps a wide field awaiting investigation along such lines—a field embracing such widely separated conditions as fibrositic rheumatism, some psychoses, and neuropathies."

In connection with Stannus's attribution of generalised tissue anoxia to riboflavine deficiency it is of interest and of some importance to recall two points :—

1. Riboflavine, as a prosthetic group, plays a part in both ærobic and anærobic oxidations in the body, the former in the vascular tissues and the latter in the avascular tissues, notably the eye.

2. There is an increasing tendency to attribute anæsthetic (*e.g.*, ether) convulsions to hypoxia or anoxia, the incidence of convulsions being greatest in patients with heightened metabolism (*e.g.*, children), in whom the ribo-

flavine requirement is high, and in obese patients, in whom the intake or absorption of the vitamin may be deficient.

Oxidations involving every cell in the body are dependent on a sufficiency of riboflavine, so that eventually the whole body must be affected by lack of the vitamin. Long before this state develops, however, lesions are produced in the tissues whose metabolism is particularly high. Stannus suggests that the lesions should be expected to appear first in the capillaries and then in the tissues immediately surrounding them. Capillary dilatation will be the first symptom, followed by impaired nutrition and function of the tissues.

Capillary dilatation is not ordinarily observable except in the eye, and in this situation it has been the subject of considerable controversy, particularly from the aspect of its ætiology. In the tongue the dilatation, together with sluggish blood-flow, causes a glossitis in which the tongue is magenta in colour. This is regarded as a significant diagnostic feature, and is to be differentiated from the scarlet tongue characteristic of nicotinic acid deficiency. Many factors may cause increased vascularity of the cornea, and this may not be a reliable sign of deficiency in any one patient. It may provide confirmatory evidence in conjunction with other symptoms, and when a large group of patients is considered, a high incidence is of definite significance. This is the conclusion of Lyle, Macrae and Gardiner (*Lancet*, March 25, 1944, p. 393) following the examination of nearly 4,000 R.A.F. subjects of varying nutritional status. They are of the opinion, however, that there is some other substance in addition to riboflavine which is partly responsible and which is present in oranges and vegetables generally.

Lyle *et al.* (*ibid.*) describe four degrees of corneal vascularity which correspond to the various degrees of deficiency of riboflavine and the unknown factor :—

Type A. A few small vascular " twigs " in the limbus

Type B. Increased vascularity of the limbus with anastomosis of the " twigs "

Type C. As type B but vascular " twigs " extending into the clear cornea

Type D. As types B and C but with anastomosis of the " twigs " in the cornea forming vascular loops

Type A is probably of no significance, but the other three types, if occurring in conjunction with other signs of nutritional deficiency, may be indicative of deficiency of riboflavine or an unidentified " vascularity factor " or of both.

Next in order of appearance as signs of riboflavine deficiencies are the various forms or degrees of stomatitis, an important description of which was given by Jones, Armstrong, Green and Chadwick (*Lancet*, June 3, 1944, p. 720), although they did not observe the magenta-coloured tongue reported by other observers. The lesions they observed, in order of appearance and of increasing importance as indications of deficiency, are as follows :—

> Soreness of the tip and/or edges of the tongue with redness and decreased conspicuousness of the papillæ. " As the condition progressed the change spread backwards in a triangular pattern with the base of the triangle at the tip and the advancing apex central and pointing towards the root of the tongue."
>
> Decrease in redness of the tongue with enlargement of papillæ, which become " mushroomed " or flat-topped. Filiform papillæ appear to turn white, and the fungiform papillæ develop a bright-red centre.
>
> Increasing smoothness of the tongue and appearance of fissures about 1 mm. deep or shallow ulcers.
>
> Finally complete atrophy of the surface of the anterior end of the tongue with wasting and a sharp rather than rounded edge to the tongue.

At some stage during the development of the symptoms in the tongue the lips and cheeks may become involved (" angular stomatitis "). The first lesion noted by Jones *et al.* (*ibid.*) was " a tiny, painful, raw, red area at the commissure of the lips where the mucous membrane joins the skin. The red area spread along the mucosa of the cheek and lower lip, but tended to be confined to a size of less than a square centimetre. Quite early the reddened patch became covered with white epithelium, looking like sodden blotting paper, which could not be scraped off easily. This white coating sometimes extended to form quite large sheets which might be criss-crossed by cracks, giving the appearance of sun-

dried mud which had flaked. Concurrently, small fissures often developed in the skin at the corners of the mouth; they were apt to be very painful."

The principal remaining symptoms of hyporiboflavinosis are neurological. The "capillarity" of nerve-tissues, like other tissues, is in direct proportion to their metabolic "activeness" and to their susceptibility to degeneration following riboflavine deficiency. The clinical symptoms of the neurological lesions are mainly sensory according to Stannus (*Brit. Med. Journ.*, July 29, 1944, p. 140), and they include "a sense of muscular weakness, inco-ordination, ataxia, and paræsthesia—unassociated with any real loss of power, any loss of sensation, or any muscular wasting—together with loss of visual and auditory acuity." Some of these symptoms have been referred to as part of a syndrome of peripheral neuritis and due therefore to a vitamin-B_1 deficiency. Stannus attributes them to a hyporiboflavinosis, but suggests that some other factor may be involved, presumably some as yet unidentified factor of the vitamin B_2 group.

Craigie's figures indicating the relative degrees of capillarity of various parts of the nervous system are quoted by Stannus, who regards them as significant and as offering an explanation of the localisation of the characteristic group of neurological symptoms.

Clinical Uses and Doses of Vitamin B_2

From a consideration of the physiological action of vitamin B_2, some idea of its clinical uses can be inferred, but there are other conditions not so far mentioned and which are not obviously related to "capillary dysergia", but which have been reported to respond to vitamin B_2. Some such symptoms are included in this section, together with individual symptoms which may be included in Stannus's syndrome of "capillary dysergia".

(In conformity with accepted usage and the official nomenclature, vitamin B_2 will be referred to hereafter as riboflavine.)

Prophylactically riboflavine is probably best administered collaterally with other members of the vitamin B group, either the whole group in the form of yeast, yeast extract,

unrefined liver extract or simply by inclusion in the diet of an increased proportion of the foods richest in vitamin " B ". These last include lean meat, liver, kidneys, sweetbreads, eggs and milk. Alternatively vitamin B_1 and nicotinic acid may be given with riboflavine.

Preliminary indications for prophylactic use of riboflavine can only be deduced from a consideration of the patient's diet, from which an incipient deficiency may be deduced. At a later stage similar evidence may be supported—visible signs such as increased corneal vascularisation, stomatitis, soreness of the tongue, ptyalism, etc. According to Sydenstricker, proliferation of vessels of the limbic area with loops penetrating the cornea in the segment from " 5 o'clock " to " 7 o'clock " is indicative of deficiency, but similar lesions in the segments " 2 o'clock " to " 5 o'clock " and " 7 o'clock " to " 10 o'clock " cannot be accepted as definite indications of deficiency.

Dosage of riboflavine is still to some extent arbitrary. Officially, the dose is given as 1 mg. to 10 mg. (B.P., Sixth Addendum, 1943). The daily dietary requirement of an average adult has been accepted as 2·5 mg. to 3 mg., but it may be that considerably more than this is absorbed from the intestine from that synthesised by the intestinal bacteria. During administration of sulphonamides and other drugs which sterilise the bowel contents it is advisable to give prophylactic doses of the order of 10 mg. Other patients who have some indication of deficiency, or in whom a dietary deficiency is suspected, may be given 2 mg. to 6 mg. daily.

Doses of 10 mg. of riboflavine may be given intramuscularly daily for several days as a precautionary measure for patients who might be expected to be subject to ether convulsions (see page 216).

Therapeutically riboflavine will usually be indicated for the correction of perhaps two or three deficiency symptoms and as a means of preventing the development of further symptoms in the same patient.

The effectiveness of riboflavine in all the conditions in which it has been suggested has by no means been established. In view of its entirely non-toxic nature, however, its use may well be considered whenever there is a possibility of some

benefit resulting, especially in conditions in which other forms of treatment are not entirely successful.

For the treatment of manifest deficiencies or acute conditions doses of 3 mg. to 5 or 10 mg. should be given daily. The oral route of administration is generally satisfactory, but in cases of gastrointestinal disease (achlorhydria, diarrhœa etc.) and in hepatic disease intravenous or intramuscular injection may be necessary.

The conditions in which riboflavine is indicated or has been suggested therapeutically may be divided into three dosage groups as follows :—

Group I, 1 mg. to 3 mg. daily :—

Burning and itching of eyelids
Lachrymation
Photophobia
Twilight blindness

Group II, 3 mg. to 5 mg. daily :—

Angular stomatitis
Bedsores
Circumcorneal injection
Cheilitis (= cheilosis or angular stomatitis)
Corneal vascularisation
Dermatoses
Glossitis
Keratitis, superficial
Pellagra (collaterally with nicotinamide)

Group III, 5 mg. or more daily :—

Amblyopia, tropical, nutritional
Cataract
Corneal opacities
Corneal ulcer
Keratitis, interstitial
Keratitis, rosacea
Pemphigus
Sprue
Steatorrhœa
Toxic effects of gold

Hypervitaminosis-B_2

No toxic effects have been reported following intensive treatment with riboflavine, and on a basis of animal experiments it has been estimated that no toxic effects would follow the ingestion of 20 grammes of riboflavine in a single dose. The development of hypersensitivity to riboflavine, similarly, is not to be anticipated, no cases having been reported.

CHAPTER XXII

NICOTINAMIDE

NICOTINAMIDE is the officially approved and generally accepted name for the substance formerly referred to as the pellagra-preventing factor, PP factor, vitamin PP, or vitamin B_7. Nicotinic acid, which is commonly employed as a " pellagra-preventing " factor, should be regarded as a precursor of the vitamin itself.

Historical Note

Nicotinic acid was first prepared in 1867, long before it was realised that it was of nutritional significance. Goldberger, in 1915, realised that pellagra was a result of wrong diet, but it was not until 1924 that Goldberger and Tanner postulated a definite pellagra-preventing factor. Two years later it was found that the thermostable fraction of the vitamin-B group (" vitamin B_2 ") possessed pellagra-preventing activity, and, on the assumption that this consisted of one substance only, it was erroneously identified with Goldberger's PP factor. Failure to differentiate between chick, rat and human pellagra resulted in considerable confusion in the nomenclature of " vitamin B_2 ", and probably delayed the discovery that the so-called vitamin was in fact a mixture of several substances. Eventually it was shown that human pellagra and black-tongue in dogs were analogous conditions, and this aided in the research, which culminated in 1937 in the isolation of nicotinamide from liver concentrates and the demonstration of its curative effect in canine black-tongue (Elvehjem *et al.*). The curative effect of both nicotinic acid and of nicotinamide in the major symptoms of human pellagra was then quickly demonstrated and confirmed by a number of workers, in particular Harris (1937) and Spies (1938).

Funk, in his search for the antineuritic factor, had isolated nicotinic acid in 1911, but he failed to recognise its nutritional significance.

Nicotinamide was shown to be a constituent of the molecule of coenzyme, and thereafter the chemistry of coenzyme activity was gradually elucidated.

Chemistry of Nicotinamide and Nicotinic Acid

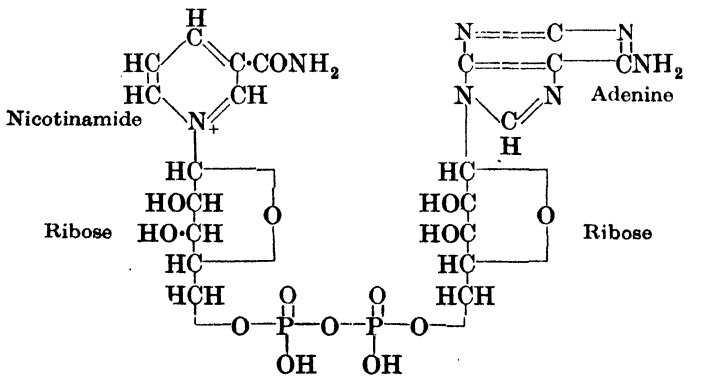
Nicotinic acid is 3-pyridine carboxylic acid (or pyridine-β-carboxylic acid). After ingestion the acid is converted into its amide, apparently in the erythrocytes, and it is the amide which is to be regarded as the vitamin and the acid as a precursor.

A number of other substances related to nicotinamide may be regarded as precursors of the vitamin, for they can be utilised as pellagra-preventing factors. These other " precursors " or alternatives to nicotinic acid include quinolinic acid (2 : 3-pyridine dicarboxylic acid), pyridine-β-carboxylic acid diethylamide (also known as diethylnicotinamide, nikethamide [B.P.], " Anacardone ", " Coramine ", " Corvotone ", or " Nicamide "), pyrazine monocarboxylic acid and pyrazine dicarboxylic acid.

It is of interest to note that quinolinic acid, although active in human pellagra, is inactive in canine black-tongue.

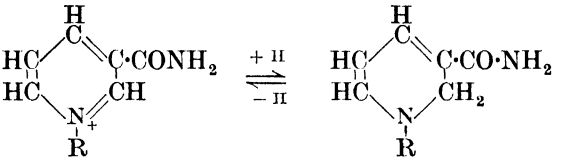
The vitamin activity of nicotinamide is exerted only after it has been incorporated into the coenzyme molecule containing either two or three phosphoric acid radicles (coenzyme I and coenzyme II respectively). Coenzymes are hydrogen-carriers, and the active atom in this process is the nitrogen in the pyridine ring.

The structure of coenzyme I is as follows :—



The active point in coenzymes I and II is the nitrogen atom in the ring of nicotinamide. Coenzyme II is a hydrogen acceptor to the enzyme oxidising hexosemonophosphate and to the citric and phosphohexonic enzymes. Coenzyme I is inactive for these enzymes, but it is active in the enzyme systems oxidising lactic, malic, β-hydroxybutyric, triose, phosphoric and α-glycerophosphoric acids and glyceraldehyde.

The reactions of the coenzymes may be represented as follows :—



Nicotinic acid is official in the British Pharmacopœia (Fourth Addendum), and so also is nicotinamide (Sixth Addendum). The acid is stable under all ordinary conditions, and no special precautions are necessary in storing it. It is soluble in 1 in 75 in water at 15° C., readily soluble in boiling water and boiling alcohol and almost insoluble in ether. The sodium salt is more soluble than the free acid and is almost invariably used for administration by injection.

Nicotinamide is soluble in 1 in 1 of water, 1 in 1·5 in 95 per cent. alcohol and slightly soluble in ether. It is soluble in 1 in 10 of glycerin and has a melting point of 128° to 131° C. Nicotinamide is best kept in well-closed containers, but no other special storage conditions are necessary.

Mode of Action of Nicotinamide and Nicotinic Acid

Before nicotinic acid can exert its vitamin activity, as has been stated previously, it must be converted into nicotinamide. Nicotinic acid, as such, therefore has no vitamin activity.

The rôle of nicotinamide as principal prosthetic group in coenzymes I and II has already been indicated in the previous section on the chemistry of the vitamin. It might be expected that, since a closely related part of the oxidative breakdown of carbohydrates is involved in both instances, symptoms of deficiency of nicotinamide and of riboflavine would prove to

be practically identical. There are certain resemblances, but the differences are even more marked, and are such as cannot yet be explained in physiological terms.

It is probable that most, if not all, vitamins act intracellularly, and that whatever vitamins are required in the metabolic processes of any particular cell, these vitamins are present in this cell under normal conditions. That is to say, there is no " action at a distance ", nor is an intermediate metabolite in a process involving vitamin activity passed from a cell lacking a particular vitamin to a neighbouring cell containing it in order to complete a further stage in metabolism. This being so, it is not easy to see why tissues lacking riboflavine should show deficiency symptoms different from those shown by the same tissues lacking nicotinamide, for deficiency of either vitamin means that the same processes are inhibited at virtually the same point.

Until such questions as these can be answered, it must be admitted that the mode of action of these vitamins is only imperfectly understood.

Physiological Effects of Nicotinamide Deficiency

Pellagra, the classical condition produced by nicotinamide deficiency, is concisely stated to be characterised by " the three D's "—dermatitis, diarrhœa and dementia. Thus pellagra resembles hyporiboflavinosis in so far as the primary lesions in both conditions appear in the same or in adjacent tissues, the skin, " alimentary " mucous membranes, and the tissues of the nervous system.

It is possible that the symptoms of hyporiboflavinosis and " hyponicotinamidosis " have not yet been completely differentiated, first, because clinical deficiencies are almost invariably of both factors; second, because the lesions in both states possess certain similarities; and, third, because the original classical descriptions have inevitably not been based upon clearly defined clinical disease entities. It is unfortunate that pellagra has become so closely associated with nicotinamide deficiency, for, although nicotinamide deficiency is the predominant deficiency, there are others which almost invariably occur collaterally. The " subpellagrous " deficiency states (" hypovitaminoses-B ") most commonly encountered are

mainly of nicotinamide, apart from those in which hypovitaminosis-B_1 predominates.

The symptoms of nicotinamide deficiency may be conveniently considered in three groups, each group representing a progressively increasing degree of severity of deficiency and of resultant symptoms.

1. Group of symptoms of impairment of nervous and mental function without demonstrable organic lesions :—

 Apprehension, lassitude, slight mental retardation, confabulation and amnesia for recent events

2. Group of symptoms of early organic lesions with more marked nervous symptoms :—

 Paræsthesiæ, vertigo, headache, insomnia, depression and mild delusion states (without marked loss of insight)

3. Ultimate group of marked somatic lesions with psychotic symptoms indicating degeneration of nervous tissues :—

 Glossitis, dermatitis, and diarrhœa (with gastro-intestinal lesions), accompanied by marked disorientation, mania, profound depression, hysterical and confusional episodes and paranoia

There are some variations in the relative intensities and order of appearance of these symptoms, the variations being controlled to some extent by the acuteness or "chronicity" of the deficiency. As would be expected, impairment of nerve function predominates in acute deficiencies, since lack of the vitamin impairs function before evidence of structural change becomes manifest. If an acute deficiency is particularly severe, confusion, delirium, hallucination and mania predominate.

It has been noted that sulphonamides and other drugs which bring about a marked decrease in the bacterial flora of the intestine affect the production of riboflavine more than that of other members of the vitamin B group. Production of nicotinamide is perhaps affected to a considerable extent,

however, and this may account in part for the depressing effect of these drugs. For this reason nicotinic acid has been suggested for administration as a means of preventing such undesirable effects.

The somatic lesions of nicotinamide deficiency are characteristic to a considerable degree, but the physician should be thoroughly familiar with them in order to differentiate them from the lesions resulting from related and complicating deficiencies. Ocular lesions have not been reported, as they have been from riboflavine deficiency, although the pupils may be dilated and the sclera a bluish or leaden colour, and lesions of the buccal mucous membrane are generally limited to the tongue and the gingival mucosa. The glossitis is said to be distinguishable from that of hyporiboflavinosis by the scarlet as distinct from the magenta colour of the tongue. This has been questioned, but if there is no indication of actual or incipient cheilosis (" angular stomatitis "), hyporiboflavinosis can generally be excluded.

Before a typical dermatitis appears, the complexion may be muddy, and a slightly pigmented or macular eruption may appear on the face. Later the characteristic dermatitis appears, usually bilaterally symmetrical. It appears on those areas exposed to light or subject to minor trauma or irritation, (especially from body secretions), such as the face, neck, hands, forearms, sternum, labia, anus, scrotum and shins. At first the lesions are erythematous and to some extent resemble sunburn. Later they change to a reddish-brown colour, and begin to desquamate, the lower skin becoming thickened. At a later stage the skin may be smooth and shiny, with a roasted appearance, or it may become cracked and present a " crazy-paving " appearance. Finally, in chronic cases, hyperkeratosis may appear, followed by exfoliating dermatitis. Other skin lesions appear less frequently, or they may be absent, as in the so-called " pellagra sine pellagra ". In this last condition it is possible that there is a considerable degree of hyporiboflavinosis, although it has been stated that the skin lesions are characteristically absent in northern and temperate regions.

Ulcerative stomatitis and marginal gingivitis (which may be due in part to hypovitaminosis C) reduces resistance to

fuso-spirochætal infection, and Vincent's angina ("trench mouth") may readily result.

Clinical Uses of Nicotinamide and of Nicotinic Acid

Nicotinic acid, in consequence of its non-vitamin vasodilator action, has been employed in the treatment of angina pectoris and of asthma. In angina pectoris it seems to be most effective when given in fairly dilute solution by intravenous injection. The method was described by Neuwahl (*Lancet*, Oct. 10, 1942, p. 410). Six injections are given, each containing 100 mg. to 300 mg., as an intravenous drip infusion of a 0·05 per cent. solution of nicotinic acid in isotonic solution. Two infusions are given weekly, and after treatment patients are reported to be free from anginal attacks for at least three to six months. Blood pressure and heart rate are reduced.

The vasodilator effect of nicotinic acid is exerted after oral administration, so that it is reasonable to expect that the beneficial effects of a series of injections would be augmented and prolonged by the administration of suitable oral doses. Indeed, before the appearance of Neuwahl's paper, nicotinic acid had been recommended in oral doses of 50 mg. to 100 mg. daily for the relief of angina pectoris (*Lancet*, May 23, 1942, p. 633). Other conditions in which it has been suggested that the vasodilator action of nicotinic acid might be of value include asthma, trigeminal neuralgia, spastic bronchitis, Ménière's disease and peripheral vascular diseases. It has been reported to be of some value in Korsakoff's psychosis and cerebral thrombosis, and in these conditions it may be that the vasodilator action is the important factor.

In addition to its uses in the conditions so far mentioned, nicotinic acid may also be employed in those conditions mentioned in the succeeding paragraphs as indications for the use of nicotinamide. In this latter group of conditions, however, it is becoming more and more the practice to use nicotinamide in order to avoid the flushing of the face and other body surfaces, to which many patients may object. Subsequent references to nicotinamide may therefore be understood to include nicotinic acid unless the contrary is stated.

The principal conditions in which nicotinamide is indicated have already been enumerated on page 227 in three groups.

It will be realised that any one of these conditions, considered alone, might be caused by a number of factors. It is important, therefore, for the physician to look for some accompanying lesion or neurological derangement, and also to consider the patient's diet, and from this evidence to make a decision as to the probability of a nicotinamide deficiency. If this is not done, and if nicotinamide is prescribed on insufficient evidence of deficiency, disappointment will result. Usually in cases of definite nicotinamide deficiency a whole syndrome indicative of such a deficiency will be found, and in any one patient this is likely to include both mental and somatic symptoms. For example, a patient may have glossitis, ptyalism, paræsthesiæ and amnesia for recent events. These symptoms may be attributed to nicotinamide deficiency, and if the diet is rich in carbohydrates and fats but deficient in proteins the diagnosis is practically certain. The picture may well be complicated, however, by associated deficiencies, and there may be complaints of vague neuritic pains, cheilitis, constipation and lassitude, suggesting deficiency of aneurine and riboflavine. Almost invariably deficiency of any one vitamin of the B group will be accompanied by deficiencies of one or probably more of the others. Thus diagnosis becomes a matter of recognising a number of complicating deficiency symptoms and of deciding which specific deficiency predominates. Thereafter treatment will consist of the correction of the principal deficiency and of prescribing collateral treatment or improvement in the diet as a means of correcting the collateral deficiencies.

Menorrhagia and metrorrhagia may not be suggestive of a hypovitaminosis B, but resistant cases which do not respond adequately to the usual hormonal treatment with progestin and chorionic gonadotropin should be considered from the point of view of being caused by a vitamin B deficiency. The liver is the principal organ concerned in the inactivation of the œstrogenic hormone, and its efficiency, in this respect particularly, may be impaired by a deficiency of vitamin B. Progestin and the androgens continue to be inactivated normally in such patients, and so the œstrogen excess is not counterbalanced by the normal " antiœstrogens ", and the menorrhagic tendency tends to be accentuated and prolonged. In addition, such a " hyperœstrogenic " state tends to cause

mastitis characterised by premenstrual pain and nodularities from epithelial hyperplasia. Marked relief of this condition of menorrhagia or mastitis, or both, has been reported following a diet rich in vitamin B, together with a series of six to eight injections (two injections per week) of a solution providing aneurine 10 mg., riboflavine 4 mg., pyridoxine 10 mg., calcium pantothenate 5 mg., and nicotinamide 150 mg. in each injection (*Manitoba Med. Rev.*, May 1945, p. 191).

As will be seen from the discussion of the effects of nicotinamide deficiency, almost all cases are likely to exhibit neurological symptoms. The chronic deficiency states may be indicated by neurasthenia in mild cases to frank pellagra in severe deficiencies. Acute severe deficiencies are likely to be characterised by toxic confusional states or stuporose states of the encephalopathic syndrome of Jolliffe. The toxic confusional states are generally a result of surgical operations, labour, or any debilitating illness. Restricted diet or the increased metabolic demands brought about by fever seem to be the precipitating causes, and the administration of considerable quantities of glucose may accentuate the condition by exhausting the depleted reserves of nicotinamide. A short period of confusion may be the first indication of the deficiency, but this stage may not appear, and the deficiency may become manifested, without previous warning, by an acute toxic confusional state, with hallucinations, irrationality, and marked psychotic derangement.

The response to the administration of nicotinamide in adequate doses is sudden and dramatic. The patient may become rational and normal in behaviour in a matter of twelve to twenty-four hours.

Dosage of Nicotinamide and Nicotinic Acid

The daily requirement of nicotinamide may be supplied in the form of an equal weight of nicotinic acid, but what this weight is cannot yet be regarded as finally settled. The dietary requirement has been estimated as being of the order of 10 mg. to 30 mg. daily. Such estimates have been based on the assumption that the only source of nicotinamide is the diet. It is now known, however, that a considerable proportion of the daily intake must be provided from that synthesised by

intestinal bacteria. Just what proportion is provided from this source is not known, and it is a matter of considerable difficulty to discover this. The total amount synthesised in the intestine is certain to vary within quite considerable limits. The bacterial content of the intestine may vary, the vitamin-producing activity will not be constant, and the frequency of bowel motions will have a considerable effect as well as the fæcal consistency. One suggestion is that 80 per cent. of the total requirement may be provided by the intestinal bacteria, but much more evidence must be provided before an approximately accurate estimate can be made.

Another complicating factor is that anærobic bacteria tend to destroy or inactivate nicotinamide. Thus it will be necessary to have some knowledge of the relative proportions of ærobic and anærobic bacteria normally present in the bowel and of the approximate rate at which the nicotinamide is synthesised by the one group and inactivated by the other.

The time has not yet come, however, to reject entirely the suggested daily dietary requirements of nicotinamide, nor of any of the other vitamins known to be synthesised in the bowel —that is, produced " symbiotically ". It is suggested, therefore, that 30 mg. should be accepted provisionally as a generous estimate of the daily dietary requirement of nicotinamide. Further, this may also be regarded as a satisfactory prophylactic dose for administration as an extra-dietary supplement to prevent the appearance of deficiency symptoms when it is suspected that the diet is not adequate or when extra metabolic demands may temporarily increase the requirement.

Nicotinamide may be regarded as being non-toxic, so that it is always safe, and perhaps generally advisable, to give generous doses in treatment.

The official doses of nicotinic acid are given as 50 mg. to 100 mg. (B.P., Fourth Addendum) and those of nicotinamide as 20 mg. to 100 mg. (B.P., Sixth Addendum). These may be regarded as rather small average doses for treatment, and should the physician consider it desirable, or should the deficiency state be severe, much larger doses may be given unhesitatingly. In practice the usual maximum dose may be regarded as 500 mg. to 600 mg., although there is no objection to giving as much as 1 gramme daily. When giving doses of 100 mg. or

more it is generally advisable to give nicotinamide rather than nicotinic acid, in order to avoid the flushing effect. This does not apply, of course, when the non-vitamin vasodilator effect of nicotinic acid is required, as in angina pectoris and asthma.

It is unnecessary to give individual doses of nicotinamide for each specific deficiency symptom, and the indications are therefore given below in four groups, according to the degree of deficiency from which they arise.

Co-operative patients who are able to retain liquids are generally given nicotinamide orally, injection (usually intravenous) being employed only in stuporous or delusional patients, who may be anæsthetised if necessary as a preliminary to giving the injection.

Although individual symptoms are given in the following groups, it must again be pointed out that nicotinamide should not be given when only one such symptom is present in a patient, but rather when a group of several make it reasonably certain that nicotinamide is actually indicated. Further, the diet of all patients should be such as will provide as much of the other members of the vitamin B group as possible as soon as the patient is able to take a reasonable diet.

Amnesia for recent events Apprehension Confabulation Lassitude Mental retardation, slight	50 mg. of nicotinamide one to three times daily
Headache Insomnia Ménière's disease Paræsthesiæ Vertigo	50 mg. of nicotinamide two to four times daily
Angina (Vincent's) Delusional states, mild Depression Dermatoses, pellagrous Diarrhœa Gingivitis Glossitis	50 mg. or 100 mg. of nicotinamide at intervals up to a total of 300 mg. or 400 mg. daily

Confusional states, toxic Delusional states, acute Dementia Hallucination Pellagra, acute	100 mg. to 200 mg. of nicotinamide repeated up to a total of 600 mg. or even 1 gramme daily

Angina Pectoris.—In mild cases, or as continuation treatment after intravenous injection or infusion in severe cases, 30 mg. to 50 mg. daily are suggested to give relief or to prevent the recurrence of severe attacks. Severe cases should be given 100 mg. to 300 mg. twice weekly for three weeks. Each dose may be given in the form of an intravenous infusion of 0·05 per cent. isotonic solution. Alternatively, slow intravenous injection of a well-diluted solution may be given if intravenous infusions are impracticable.

Asthma.—As in angina pectoris, oral administration (perhaps 25 mg. two to four times daily) may prove to be of value in averting mild attacks or in decreasing their frequency or mitigating their intensity. It has been reported that a dose of 50 mg. of nicotinic acid given intravenously or 100 mg. intramuscularly gives rapid relief of a developing asthmatic spasm and freedom from subsequent attack for periods of half an hour to several days.

Ménière's Disease.—Intensive treatment is necessary and daily doses of 250 mg. given orally have been recommended. Administration must be continued for two to three months, and vitamin B_1 should be given collaterally in doses of 20 mg. daily.

Contraindications and Overdosage

Patients may occasionally be found who appear to be somewhat more than normally susceptible to the vasodilator action of nicotinic acid. The flushing normally produced may be accompanied by irritation or tingling, particularly noticeable on the ears. It is possible that the highest incidence of such reactions will be found among patients who are not deficient in the vitamin and in whom the conversion of nicotinic acid into its amide may be abnormally slow in consequence.

Apart from this, reactions to nicotinic acid have not been reported, and none whatever have been reported to nicotinamide. A large dose of nicotinic acid given to a deficient patient may cause transient flushing, and smaller doses may be expected to produce it in patients with little or no deficiency.

There are no known contraindications to nicotinic acid or its amide.

CHAPTER XXIII

THE "FILTRATE FACTORS" OF THE VITAMIN B GROUP

THE three principal members of the vitamin B group, vitamin B_1, riboflavine and nicotinamide, are of primary importance in human metabolism and are known to be essential. The other members of the group, which will be considered in this and the two following chapters, are known to be essential growth factors for many organisms. It has been suggested that they are only indirectly essential for humans, in that they only ensure the growth and normal activity of the intestinal bacteria which provide a considerable proportion of the amounts of the three primarily important factors. This hypothesis is by no means generally accepted, but it may be partly true, and it may eventually be shown that there is an exchange of growth factors between various intestinal organisms (symbionts) and between these organisms and the host (?), who must also be regarded as one of the symbionts in the partnership.

The secondary factors in the vitamin B group may be classified in various ways, but it is convenient here to deal with them in three groups: the "filtrate factors" (considered in this chapter), the adsorbate factors (Chapter XXIV) and a group of miscellaneous unclassified factors (Chapter XXV) which may eventually be shown to be associable with the factors in either the filtrate or adsorbate groups.

If an aqueous extract of yeast is treated with fuller's earth, vitamin B_1 and riboflavine are adsorbed. Further treatment of the filtrate with fuller's earth results in the adsorption of pyridoxine, adenylic acid and other factors, together constituting the adsorbate factors (see Chapter XXIV). The filtrate contains the group of vitamins known collectively as the filtrate factors. Much is known about some of these, others are not well known, and there may remain a number yet to be identified.

The rôle of most of the filtrate factors in human metabolism is by no means fully understood, and, as has been stated, it is

not yet certain if all of them are essential factors in human, or even in mammalian, metabolism. Many of these factors have been used clinically and experimentally, however, and some physiological properties have been attributed to them.

Filtrate Factors	Adsorbate Factors	Other Factors
p-Aminobenzoic acid	Riboflavine	Biotin
Pantothenic acid	Pyridoxine	Folic acid
Inositol	Nicotinamide	Vitamin M
Choline	Adenylic acid	Xanthopterin
Vitamins L_1 and L_2		

p-Aminobenzoic Acid

p-Aminobenzoic acid was synthesised in 1863 by Fischer by reducing 4-nitrobenzoic acid with ammonium sulphide. The pure substance occurs in colourless needles, freely soluble in alcohol and boiling water, and in 1 in 200 in water at room temperature.

Esters of *p*-aminobenzoic acid are well known as efficient synthetic local anæsthetics, particularly procaine, but the vitamin activity of *p*-aminobenzoic acid was not recognised until Ansbacher showed that it was necessary for normal pigmentation of the hair of the rat and as a growth-promoting factor for the chick (*Science*, 1941, **93**, 164).

The vitamin has been observed to have an absorption band which is particularly effective over the range (*circa* 3000 Å) which is most active in producing sunburn.

It now seems to be generally agreed that the antibacterial properties of sulphanilamide are due mainly to the fact that this substance supplants *p*-aminobenzoic acid in the metabolic processes of bacteria, and so brings about an arrest of the bacterial metabolic processes and prevents growth and reproduction of the bacteria, if not their death. It has been suggested, therefore, that *p*-aminobenzoic acid might have an antisulphanilamide effect, and that its administration during treatment with sulphonamides would inhibit the antibacterial effect. Such an effect is produced at or below normal body temperature, but in pyrexial patients (temperature above 37° C.) it seems that not only does *p*-aminobenzoic acid exert

no antisulphanilamide effect but it actually acts as an antibacterial and augments the action of the sulphonamides (Ansbacher, "Vitamins and Hormones", Vol. II, p. 234). Further, the action of sulphadiazine in protecting mice against meningococci is inhibited by *p*-aminobenzoic acid only if the latter is present in considerable concentration for prolonged periods. On the whole, therefore, it seems that ordinary doses of *p*-aminobenzoic acid are not likely to interfere seriously with sulphonamide treatment, especially in pyrexial conditions. When the sulphonamide is applied locally and the *p*-aminobenzoic acid is given systemically, the concentration of the sulphonamide in the infected area is likely to be such as to overcome the possible neutralising action of the vitamin.

In practice it is considered advisable to avoid the use of massive doses of procaine or related local anæsthetics in patients who are likely to need treatment with a sulphonamide within a few hours. Amethocaine is a suitable local anæsthetic for use under such circumstances.

p-Aminobenzoic acid exerts antioxidant effects, and appears to inhibit the toxic effects of arsphenamines and organic antimony compounds without impairing their antispirochætal and antiprotozoal properties.

It has been suggested that *p*-aminobenzoic acid *per se* is not a vitamin because it must be given in amounts in excess of those usually provided in the diet before it produces demonstrable experimental or clinical effects. It must be pointed out, however, that it may be a precursor of the actual vitamins, for there are much more active compounds which might be formed from it in the body. *p*-Aminobenzoyl-*l*-glutamic acid is an example, although this particular substance is not known to occur naturally. The precise rôle of *p*-aminobenzoic acid in the body is not known, but it seems likely that it acts as a coenzyme to phenolase. This seems to be confirmed to some extent by the part it appears to play in maintaining the normal degree of pigmentation of hair. In addition, *p*-aminobenzoic acid is among the many antithyroid substances mentioned by Astwood (see thiouracil, p. 31). It is also said to be an effective detoxicant for the arsenical drugs.

It is difficult to attribute precise activities to *p*-aminobenzoic acid, or to any of the other secondary vitamins of the

B group with any degree of certainty, for administration of one may stimulate the bacterial synthesis of several others in the intestine, so that confused pictures may be produced. Further, the antiachromotrichial effect of *p*-aminobenzoic acid deficiency may not be manifested if inositol, biotin and pantothenic acid are being taken in optimum amounts. Thus deficiency of hair pigment may be a manifestation of vitamin imbalance rather than of simple vitamin deficiency.

The filtrate factors generally, and *p*-aminobenzoic acid in particular, appear to be concerned in a number of the physiological processes of the liver. Inactivation of hormones, especially œstrogens, appears to be a liver function, and this is disturbed by deficiencies of riboflavine, aneurine, pyridoxine and perhaps some other members of the vitamin B group. In contrast to this, *p*-aminobenzoic acid seems to inhibit the process of hormone inactivation. In physiologically normal amounts it perhaps exerts a controlling action on this process, thus preventing unduly rapid inactivation. When the concentration of this vitamin is artificially increased following the administration of the pure substance, its effect may be physiologically excessive, so that hormone activity may appear to be potentiated by the vitamin. This may be the explanation of the reported value of *p*-aminobenzoic acid in relieving asthma (adrenaline-protecting effect) and in increasing libido (testicular hormone-protecting effect).

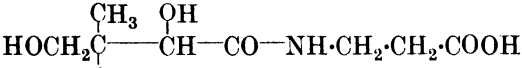
It will be evident from the foregoing that the indications for the clinical use of *p*-aminobenzoic acid are as yet by no means clearly defined.

The most general clinical use of *p*-aminobenzoic acid is for the treatment of grey hair, particularly that which has become prematurely grey. Dosage is usually of the order of 100 mg. three times daily. The oral route is invariably employed, and treatment may have to be continued for six to nine months before satisfactory results are produced.

Experimentally it seems reasonable to give *p*-aminobenzoic acid to patients who appear to show hormonal deficiencies but in whom there is no definite evidence of glandular hypoactivity to which the deficiencies could be attributed—that is to say, in patients whose hormone-inactivating function may be abnormally active.

Pantothenic Acid

p-Aminobenzoic acid and pantothenic acid are the two principal members of the group of filtrate factors. Whereas *p*-aminobenzoic acid was synthesised and its structure established before its vitamin properties were recognised, pantothenic acid was detected as a vitamin and then isolated before its structure was determined. Thus this vitamin was given its non-chemical name, pantothenic acid, by Williams in 1933 indicating its wide distribution (from pantothen—*πάντοθεν* —meaning " from everywhere "). This name has been retained as a convenient short name for general use, for the chemical name is too cumbersome for this purpose. Pantothenic acid was synthesised from α-hydroxy-β : β-dimethyl-γ-butyryl lactone and β-alanine by condensation, giving α : γ-dihydroxy-β : β-dimethylbutyryl-β-alanine, the structure of which is represented by the formula :—



This synthesis was effected by Williams and Major in 1940. Chemically this substance is something of a curiosity, being a compound of an amino-acid and a substituted butyric acid derivative.

Pantothenic acid is unstable, and the calcium salt is usually employed clinically. This is a colourless crystalline powder with a slightly bitter taste. It melts at 198° to 200° C. and is soluble in about seven parts of water at 25° C. It is stable to light and oxygen, but is slightly hygroscopic. Solutions are slightly unstable to heat, and should be sterilised by filtration and not by autoclaving.

Pantothenic acid is probably present in all foods except white flour, white sugar and similar highly refined foodstuffs. Many foods are probably poor sources, but yeast, liver and eggs are the richest.

Various investigators have noted diverse effects resulting from deficiency of factors which they did not completely identify, but which have subsequently been identified with, pantothenic acid. In consequence pantothenic acid has acquired a number of synonyms. Among these are : chick

antidermatitis factor, bios IIa, filtrate factor II, and bird antidermatitis factor. It has been suggested that it may be the so-called " spectacled-eye factor " of rats, and that it may also be the Williams–Waterman pigeon-weight factor (vitamin B_3). If pantothenic acid is identical with this last factor it may also be the " gizzard-erosion " factor.

In chicks pantothenic acid causes neuromalacia of the spinal cord as well as dermatitis. Adrenal degeneration characterised by congestion, hæmorrhage, atrophy, necrosis, fibrosis, hæmosiderosis and lack of cortical fat has been reported in rats whose diet is deficient in pantothenic acid. Since normal secretion of cortical hormone appears to be dependent on the presence of a sufficiency of fat, pantothenic acid may be necessary for the formation of suprarenal cortex hormones. In rat testes abnormal cells may appear, and there is some diminution in the number of spermatozoa produced.

Inferior pelts and hypertrophied and red-mottled thymus glands result from a deficiency in the diet of silver foxes.

These postulated pantothenic acid deficiency symptoms, it will be observed, involve derangements of the metabolism of both fats and of proteins. It is perhaps significant, therefore, that pantothenic acid combines in its molecule a relatively simple derivative of a simple fatty acid and an amino-acid. It may be that this fact will provide a clue to the elucidation of the precise nature and mechanism of its function as a vitamin.

As in the case of *p*-aminobenzoic acid, the function of pantothenic acid is as yet too ill defined to justify giving any very precise suggestions for its clinical use. The vitamin is often included in preparations of the vitamin B group, particularly in America, on the grounds that it is probably an essential factor in human nutrition, but it is to be hoped that it will soon be available in quantity for trial on a considerable scale.

Clinical trial in diseases of deranged fat metabolism appear to be worth while, particularly in sprue, cœliac disease, Gaucher's and Niemann-Pick's diseases and in the early stages of Addison's disease in an attempt to improve the secretion of suprarenal cortical hormone.

If pantothenic acid is involved in phosphorylation processes

in the body, as it possibly is, it may be worth investigation in the treatment of apparent riboflavine deficiency, in which the lesions arise from defective phosphorylation of riboflavine rather than from an actual riboflavine deficiency.

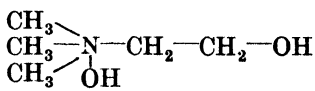
The relationship of the hæmopoietic filtrate factor of Edgar and MacCrae and the extrinsic factor (Castle) is probably well worthy of investigation from the point of view that either or both of these factors may contain pantothenic acid.

Provisionally pantothenic acid, in the form of its calcium salt, may be given in doses of 50 mg. once or twice daily. Both the oral and the intravenous routes have been employed, and there seems to be little if any difference in the activity of the vitamin by either route.

Choline

Choline was first isolated from pig's bile in 1849 by Strecker. Babo and Hirschbrunn isolated a basic substance from white mustard by hydrolysis of the alkaloid sinapin. They called this base sinkalin, but fifteen years later (in 1867) Claus and Keesé showed that sinkalin was identical with choline. In the same year Wurtz synthesised choline, although he called it névrine.

Choline is trimethyl-β-hydroxyethyl ammonium hydroxide, and its structure is therefore :—



It is a constituent of lecithin, and its acetyl derivative is of great importance in the transmission of impulses from nerve endings to muscles.

Pure choline can be produced in the form of a colourless crystalline mass. It is extremely hygroscopic, and so is generally in the form of a syrupy liquid. Choline is extremely soluble not only in water but also in alcohol. It is only slightly soluble in dry acetone and chloroform. Choline is a strong base and precipitates the heavy metals as hydroxides from aqueous solutions of their salts. Solutions of choline dissolve fibrin and prevent the coagulation of proteins. Dilute solutions may give rise to the highly poisonous related

substance neurine. Pure dry choline decomposes at 40° under reduced pressure, but the chloride does not decompose appreciably even at 180°.

In the somewhat limited clinical applications of choline, the chloride has generally been employed. This salt is readily soluble in water and in alcohol, is less soluble in acetone and carbon tetrachloride and insoluble in ether.

The interest in choline as a dietary factor began when it was observed in 1924 that pancreatectomised dogs, maintained for considerable periods on insulin, developed fatty livers. Further investigation showed that lecithin prevented the excessive deposition of fat in the livers, and eventually choline was recognised as the constituent of both lecithin and the pancreas, which was the active substance. The effect (" lipotropic ") of choline is shared by a number of other substances, and is exerted on cholesterol esters as well as on the glycerides. The amino acid methionine is particularly active in this respect, and this is the active substance in casein and egg-albumin, both of which are lipotropic. The efficacy of methionine was suggested by du Vigneaud in 1939 to be due to its providing methyl groups for the synthesis of choline. This was confirmed in 1940.

The precise significance of choline and of other " lipotropic " factors such as methionine in the processes of hepatic necrosis and cirrhosis is obscure. Dietary deficiency of proteins containing particularly high proportions of sulphur-containing amino acids may be effective in preventing massive hepatic necrosis, but not necessarily the hepatitis due to poisons or viruses which precede the necrotic stage. In these conditions methionine seems to be the important constituent of the protective proteins. Its importance is probably the result of its sulphur content. As a lipotropic factor, however, methionine is probably of importance in that it is a source of methyl groups for the synthesis of choline, the principal lipotropic factor. The sulphur content of methionine seems to be important also in the action of methionine as a lipotropic factor, but its significance remains obscure. *In vivo*, methionine probably provides methyl groups which are used in the methylation of ethanolamine—forming choline. In addition to its vitamin activity choline is of importance as a structural

element, mainly in nervous tissue, for it is a constituent of lecithin. It has been observed that "triethylcholine" (or, more correctly, triethyl-β-hydroxyethyl ammonium hydroxide) prevents the development of fatty liver as choline itself does, but it cannot replace choline in the promotion of growth—that is, as a unit in cell structure. Thus it appears that the methyl groups of choline are essential only for its lipotropic action.

Choline has two principal functions, therefore. It serves as a structural unit, especially in nerve tissue as a constituent of lecithin. In this connection it cannot be regarded as a vitamin, for a vitamin, by definition, does not enter into the more or less permanent structure of tissues. Choline is thus to be regarded as a vitamin in its lipotropic function. Its mode of action is unknown, but its methyl groups are undoubtedly of considerable importance and are almost certainly used in the synthesis of phospholipids—the transport form of the body-fats. In this function methionine may replace it entirely. Methionine contains sulphur, and this, too, is of importance, for choline, which contains no sulphur, is not active in the absence of cystine, an amino acid containing sulphur.

It will thus be understood why methionine is now coming to be regarded as a vitamin. Like choline, methionine is also a structural element in body tissues, in this instance the proteins, but it has an even more defined vitamin (lipotropic) action than choline.

Cholesterol is included among the lipoid substances over which choline exerts a controlling action. Deficiency of choline has been associated with an extreme toxic state characterised by hæmorrhagic enlargement and degeneration of kidneys as well as the liver, with thymic regression and splenic enlargement. Choline is used to some extent clinically for the prevention or the arrest of hepatic degeneration, which may be of dietary origin. It is not effective in preventing such changes when they are due to viruses, bacterial toxins or such toxic drugs as the organic arsenicals. It may be effective, however, in preventing the appearance of the massive necrosis which may follow the fatty degeneration.

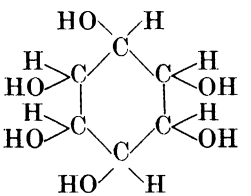
The dosage of choline has not been precisely laid down, but a suggestion for the treatment of the severer conditions is 10 c.c. of 1 per cent. solution of choline chloride given

daily by the intravenous route. More recently 2 grammes of the chloride have been suggested daily by the oral route. Larger doses (up to 6 or even 10 grammes daily) have been given but toxic effects were reported. In order to assist the choline in its lipotropic action, the diet should be low in fat content and a high protein level may be helpful.

Inositol

Inositol is a constituent of phospholipids and a member of the group of growth-promoting substances known as "bios." In 1923 bios was subdivided by Fulmer into bios I and bios II, bios I being inositol. Woolley, in 1940, identified inositol with mouse antialopecia factor, and subsequent work tends to show that the "antispectacled"-eye factor for rats is inositol.

Inositol is hexahydroxycyclohexane :—



an isomer of glucose.

The hexaphosphate of inositol is phytic acid, the supposed "decalcifying" substance in cereals. The calcium–magnesium salt of phytic acid is known as "phytin." A phospholipid from soya beans has been described and its percentage composition determined. This phospholipid, named lipositol, contains 16 per cent. of inositol, together with galactose, *d*-tartaric acid, fatty acid, phosphoric acid and ethanolamine. Similar information in regard to inositol compounds in animals does not appear to be available.

The rôle of inositol is probably similar to that of choline in so far as it is a constituent of the transport forms of phospholipids. Like choline, also, deficiency of inositol results in certain skin diseases which have not been precisely described. In rats the principal condition resulting from deficiency is a symmetrical partial alopecia. This condition can be produced in rats by administering succinylsulphathiazole, but if inositol is given collaterally the condition does not develop. In mice

inositol does not appear to be efficient in preventing alopecia in the absence of pantothenic acid. The vitamin may be concerned in reproduction and lactation, and it is said to have a marked effect in stimulating gastric and intestinal peristalsis.

Information on the clinical use of inositol is almost non-existent. Doses of 1 to 2 grammes given orally are stated to have produced good effects in " certain skin diseases " and to have produced no ill effects (*Amer. Journ. Digest. Dis.*, 1943, **10**, 45).

Vitamin L

The existence of two factors, vitamin L_1 and vitamin L_2, was postulated by Japanese workers (Nakahara *et al.*, in 1938), and a number of papers on these substances have since appeared, but their chemical nature has not been described and there is some doubt as to their existence.

The function of vitamins L_1 and L_2 is said to be the maturation of lactation tissues. More specifically both vitamins are said to be necessary for the initiation of lactation. Vitamin L_1 is of greatest importance in initiating the first lactation, although vitamin L_2 is also required. In subsequent lactations vitamin L_2 assumes the greater importance, although both are required in first and in subsequent lactations.

Vitamin L_1, or rather concentrates of it, are prepared from beef liver, and baker's yeast is the source of vitamin L_2.

The vitamins L may possibly be closely related to the lipotropic factors in function, if not chemically. Since vitamin L_1 is most important in the first lactation and only of secondary importance subsequently, it may be that it compares with choline in its structural aspect, being concerned in the development of some special tissues in the breast, perhaps a secretory endothelium of the mammary acini in the first lactation. Subsequently it may only be required to reactivate this endothelium, after which vitamin L_2 takes over the principal rôle in maintaining lactation, and perhaps performing some essential rôle in the elaboration of a specific lipid or protein of the milk.

Vitamin L is not available for clinical use in any galenical preparation, so that if deficiency is suspected it can only be corrected by dietary measures, beef liver and baker's yeast being especially important.

CHAPTER XXIV

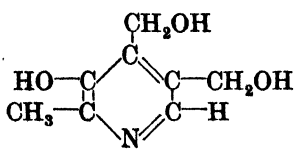
THE "ADSORBATE FACTORS" OF THE VITAMIN B GROUP

RIBOFLAVINE and nicotinamide are the principal members of this group, but they have already been considered in earlier chapters. The others are pyridoxine (vitamin B_6) and adenylic acid.

Pyridoxine

The existence of pyridoxine was reported in 1934 by György, who called it vitamin B_6. Its existence had been suspected four years earlier by Chick and Copping, who had provisionally called it factor Y. What eventually proved to be the same substance was reported as an antidermatitis factor by Hogan and Richardson in 1936, as vitamin H by Booher in 1937, and as factor I in 1936 by Lepovsky, Jukes and Krause. Isolation of the vitamin was accomplished by several workers during 1939, and its structure was determined in the same year and its synthesis was effected. The synthesis was carried out by Harris and Folkers in the U.S.A. and by Kühn *et al.* in Germany. It was Kühn who suggested that vitamin B_6 should be called adermin; this was in 1938. In 1939 György and Eckhardt suggested the name pyridoxine, and this has now been universally accepted.

Pyridoxine occurs in colourless crystals, with a melting point of 200° to 212° C. and soluble in water and alcohol. It is stable to heat and to alkalis, but is decomposed by light, particularly ultra-violet light. Pyridoxine, like nicotinamide, is a derivative of pyridine. Its constitution is described by the chemical name 2-methyl-3-hydroxy-4 : 5-di-(hydroxymethyl)-pyridine, and its structure may therefore be represented by the formula :—



Pyridoxine is stable to heat, light and acids, but it readily undergoes oxidation in the presence of alkalis. It is freely soluble in water and alcohol, but only slightly soluble in ether and chloroform. Clinically pyridoxine is used in the form of its hydrochloride, a white odourless crystalline powder with a saline taste. The hydrochloride is soluble in water (22 per cent.), in 95 per cent. alcohol (1·1 per cent.), and practically insoluble in ether. The aqueous solution is acid, having a pH of about 3. Solutions less acid than pH 6 are unstable, especially if heated, and contact with iron turns solutions red.

A number of diverse functions have been attributed to pyridoxine with varying degrees of certainty. The chemistry of its mode of action is obscure, and when this is elucidated it may do much to unify the picture of its seemingly unconnected functions and to establish their validity or otherwise.

Pyridoxine is probably essential for the normal utilisation of fatty acids. Deficiency causes fatty degeneration of the liver in rats. This is partially corrected by choline, but choline alone cannot restore the livers to normal. It has been suggested that pyridoxine is an essential factor in the synthesis of fat from protein. This, in view of its lipotropic action, suggests that pyridoxine activates a reversible enzymatic process in the synthesis and in the catabolism of fats. Pyridoxine can be partly replaced in the body by certain essential fatty acids, and it seems likely that it plays an essential rôle in their synthesis in the normal animal. These fatty acids are probably those formerly grouped together as "vitamin F", notably, linoleic, linoleinic and arachidonic acids, which have been described as "desirable constituents of phospholipids", but which are not now accepted as vitamins. Another suggestion is that pyridoxine is essential for the utilisation of unsaturated fatty acids and that the latter accumulate as a result of deficiency (*Journ. Amer. Med. Assoc.*, Aug. 19, 1939, p. 683).

Pyridoxine also has a rôle, the details of which are unknown, in protein metabolism, and is particularly associated with the amino-acid tryptophane. (See note below.)

In the association of pyridoxine with the metabolism of unsaturated fatty acids perhaps lies an explanation of its curative effect in certain neurological and dermatological

conditions and, similarly, its association with protein metabolism may provide an explanation of its action in preventing and relieving certain muscular disorders and a specific type of anæmia (microcytic hypochromic) in puppies.

The principal symptoms of pyridoxine deficiency in humans are reported to be irritability, insomnia, nervousness, vomiting, weakness and difficulty in walking (*Journ. Neurol. and Psychiatry*, Oct. 1939, p. 335).

Pyridoxine has been given collaterally with vitamin E, and the two vitamins (or pyridoxine alone) have been reported to be of value in pseudohypertrophic muscular dystrophy, arsenical neuritis, and various muscular disorders.

These must be considered to be the conditions in which pyridoxine is indicated in clinical practice. Other definite indications will probably be established in the future.

There is no international unit for pyridoxine, and doses are almost invariably indicated in terms of weight of the hydrochloride. A " rat-day " unit has been employed, and is equivalent to 10 microgrammes.

It has been suggested that the daily requirement is about 2 mg. (*Journ. Amer. Med. Assoc.*, Dec. 26, 1942, p. 1392), but this estimate is probably based on dietary intake, and does not take into account the amounts synthesised by intestinal bacteria and absorbed by the host. In cases of deficiency, therefore, it is advisable to give doses of an altogether higher order.

The vitamin is probably active orally, but it is usual to rely on the parenteral route of administration to ensure adequate and complete absorption, partly on account of the high price of the vitamin. Orally, doses of 1 mg. to 3 mg. daily have been suggested, but 10 mg. is probably a much more satisfactory minimum.

If the parenteral route is decided upon, pyridoxine hydrochloride may be given subcutaneously, although it may cause pain if given by this route. Intravenous injection is usually adopted, and this is probably much the most satisfactory route. Doses by this route are generally of the order of 50 mg. to 200 mg. Such doses may be given daily or weekly at the discretion of the physician and in accordance with the severity of the patient's symptoms.

Liver is one of the rich natural sources of pyridoxine, and liver extracts are being used to prevent or overcome the agranulocytic angina which some patients develop during treatment with thiouracil. It seems that there is some evidence that pyridoxine is the specific "antiagranulocytic" factor in liver, and doses of 200 mg. daily for three or four days are suggested for the treatment of this condition. The pyridoxine is given intravenously (*Canad. Med. Assoc. Journ.*, April 1945, p. 368). This treatment may well be worth trying in agranulocytosis due to other drugs such as the sulphonamides and barbiturates.

Recent Knowledge of Pyridoxine

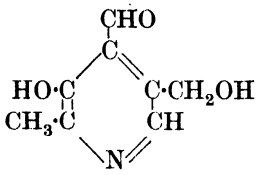
Certain derivatives of pyridoxine have now been shown to be the active forms in various metabolic processes. The phosphorylated aldehyde of pyridoxine (I), for example, is the coenzyme of tyrosine decarboxylase and of several other amino-acid decarboxylases as well as of glutamate-aspartate transaminase. The amine of pyridoxine (pyridoxamine, II) is also biologically active, but details of its function have not been fully described. The knowledge available may be summarised as follows. Interconversion of pyridoxal and pyridoxamine can be carried out by heating with amino and keto acids respectively, and it is probable that these pyridoxime derivatives function in transamination *in vivo*, themselves undergoing transamination in the process.

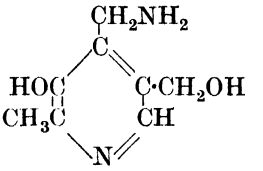
$$\text{Pyridoxal} + \text{R}\cdot\text{NH}_2\text{COOH} \rightleftharpoons \text{Pyridoxamine} + \text{R}\cdot\text{CO}\cdot\text{COOH}$$

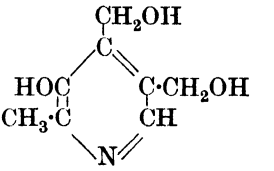
Thus pyridoxal is to be regarded as a prosthetic group in amino acid deaminases and a pyridoxamine enzyme functions as an amine donor for the amination of keto-acids. It is probable that the phosphates of the two pyridoxine derivatives are the active forms and the free bases function only after phosphorylation by adenine triphosphate. Pyridoxal phosphate, unlike pyridoxamine phosphate, is the prosthetic group in tyrosine decarboxylase.

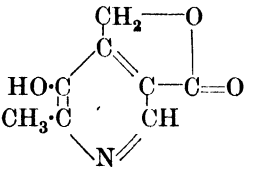
Pyridoxine (III), pyridoxal and pyridoxamine have come to be known as the vitamin B_6 group, and it is perhaps legitimate to include also the hæmopoietic and growth-promoting factors α- and β-pyracin (IV and V) in this group. The

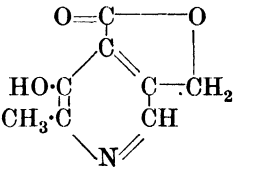
chemical relationships between these substances, all of which are pyridine derivatives, will be seen from their structures, which are as follows :


I. Pyridoxal


II. Pyridoxamine


III. Pyridoxine


IV. α-Pyracin


V. β-Pyracin

Adenylic Acid

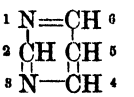
It is not universally agreed that adenylic acid is a vitamin, but there is an increasing tendency to regard it as being one. It occurs among the " adsorbed " factors of the vitamin-B group, and it is of considerable importance in certain enzymatic processes in the body which are catalysed by associated vitamins of the B group.

Adenylic acid is a constituent of the important class of nucleotides. A nucleotide consists of a purine or pyrimidine base united through a sugar molecule to phosphoric acid.

The chemistry of the nucleotides is worth considering in some detail in view of their relationship to a number of substances of biochemical importance.

The Bases in Nucleotides

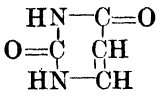
The two primary bases of nucleotides are pyrimidine and its derivative purine. The structure of pyrimidine (a unit in the aneurine molecule—vitamin B_1) is :—

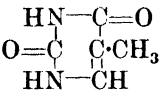


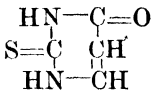
The 2 : 6-diketo-derivative of pyrimidine is known as uracil. Uracil is a constituent of yeast nucleic acid, and the

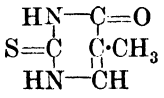
2-thio- derivative of this is thiouracil (2-thio-6-keto-pyrimidine) which has recently come into prominence as an inhibitor of thyroxine synthesis. In animal nucleic acid the substance corresponding to uracil is thymine or 2 : 6-diketo-5-methyl-pyrimidine or 5-methyl-uracil. Incidentally, it is of interest to note that methyl-thiouracil (which might be called 5-thio-thymine) has been shown to be at least as effective as thiouracil, but less toxic.

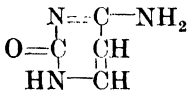
The structures of the compounds so far mentioned are shown below for comparison :—


Uracil


Thymine or methyl-uracil

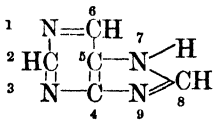

Thiouracil


Methyl-thiouracil or thio-thymine


Cytosine

The last of the above structures, cytosine, is still another of the pyrimidine bases which enters into the structure of the nucleic acids. It is 6-amino-2-keto-pyrimidine.

Biologically, some rather more complex derivatives of pyrimidine are more numerous, and possibly of greater importance. These are derivatives of the second primary nucleotide base, purine; this substance has the structure indicated by the following formula :—



The principal derivatives of purine are :—

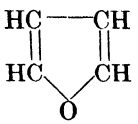
Adenine = 6-aminopurine
Guanine = 2-amino-6-ketopurine
Hypoxanthine = 6-ketopurine
Xanthine = 2 : 6-diketopurine
Uric acid = 2 : 6 : 8-triketopurine (or 2 : 6 : 8-trioxy-purine)

It will be recalled that the caffeine group of alkaloids are methylated purines.

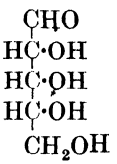
Two of the pyrimidines, thymine and cytosine, together with the purines adenine and guanine, are the four bases, the nucleotides of which, united through their sugar and phosphoric acid radicles, constitute the principal nucleic acid of animal tissues, otherwise known as thymonucleic acid.

The Sugars in Nucleotides

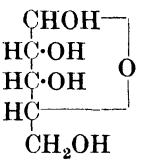
The sugars in the nucleotides are mainly derivatives of the pentose (five carbon atom) sugar ribose. In the vegetable nucleotides the sugar is the furanose form—that is, a cyclic structure related to furan. In the animal nucleotides the sugar is mostly the furanose form of desoxyribose, though both sugars occur in plants and in animals.

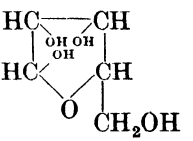


Furan

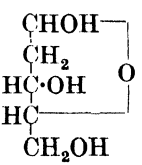


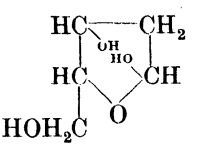
Ribose





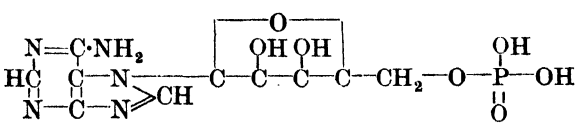
Ribofuranose





Desoxyribose (furano form)

Adenylic acid or adenine nucleotide is thus a typical nucleotide, one of several which enter into the structure of the nucleic acids. Its structure may be represented as follows :—



As so far considered, adenylic is a structural unit in the protoplasm of cells. In addition, it has an enzymatic function as a phosphoric acid carrier and it is in this sense that it is to

be regarded as a vitamin or as an enzyme. This rôle of adenylic acid (or adenosine monophosphate) is indicated in the scheme of carbohydrate metabolism on page 210. It will be seen that the phosphoric acid split off from phosphopyruvic acid is taken up by creatine and passed on to adenylic acid. Each molecule of adenylic acid takes up two molecules of phosphoric acid to become adenyl pyrophosphate or adenosine triphosphate. These two extra molecules of phosphoric acid serve to phosphorylate one molecule of hexose (fructose) which can then undergo the normal breakdown of the carbohydrates to provide muscle energy.

In most instances a vitamin is suspected in consequence of deficiency symptoms, it is isolated and its structure determined and then an attempt is made to elucidate its mode of action. Adenylic acid is an example of almost the reverse of this sequence. It was discovered and its mode of action elucidated in some detail, and from its mode of action it was considered to be a vitamin. Again, in the reverse of the usual order of events, knowing its mode of action, an attempt can be made to deduce what the deficiency symptoms may be expected to be.

It has already been noted that adenylic acid is an intermediary between creatine and fructose as a phosphate carrier. Thus it is to be expected that deficiency symptoms will arise from disturbance of carbohydrate metabolism and be manifested as derangement of muscle function. The muscles first affected appear to be those of the peripheral vascular system, and perhaps to some extent those controlling the bronchial arterioles. This is suggested from the fact that some benefit has been claimed in the treatment of Raynaud's disease and angina pectoris and related conditions with muscle extracts, which probably contain adenylic acid as active principle, and with adenosine. Adenosine may be regarded as a precursor of adenylic acid (or a provitamin), in that it is the unphosphorylated riboside of adenine. It is prepared commonly from yeast, and is therefore not identical with the corresponding predominant animal substance which is the desoxyribose compound.

It is probably unwise to speculate further than this, and a more extensive and critical clinical use of adenylic acid,

adenosine, and adenine desoxyriboside than has hitherto been carried out may yield valuable information.

Adenosine has been given in doses of 5 mg. to 10 mg. hypodermically. The corresponding desoxyriboside and its phosphorylated compound, muscle adenylic acid, may probably be given in doses of 20 mg. or even 30 mg.

CHAPTER XXV

OTHER FACTORS OF THE VITAMIN B GROUP

APART from the adsorbate factors and the filtrate factors, the vitamin-B group contains a few other factors which do not appear to fall into either of these groups. These substances will be considered in this chapter.

The most important of the miscellaneous factors of the vitamin B group appears to be biotin, a widely distributed substance remarkable for its great activity in minute amounts. The remainder of the group consists of three factors which seem to be related chemically and in their physiological function, though neither their chemistry nor their function is yet fully elucidated. These three factors are folic acid, vitamin M, and xanthopterin, all of which have some function in the process of erythrocyte formation or development.

Biotin is one of a group of factors known collectively as "bios". The term bios is becoming less and less frequently used, but it is still encountered occasionally in the literature without any precise definition being given. The constituents of bios may perhaps be enumerated here, therefore with advantage.

The name bios was given by Wildiers in 1901 to an organic material which, in small amounts, stimulated the growth of yeast. Bios is now known to consist of at least six distinct substances, all members of the vitamin B group. These substances are :—

Bios I is *meso*-inositol
Bios IIa is pantothenic acid
Bios IIb is biotin
Bios III or Bios V is aneurine
Bios IV may be β-alanine or pyridoxine

Other constituents of bios may include the plant-growth "hormones" auxin a, auxin b, and heterauxin.

Biotin

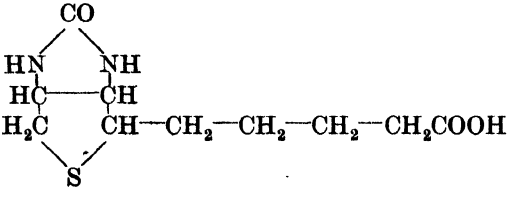
The investigation of bios IIb was undertaken by Kögl, and in 1935 he claimed to have isolated a small quantity of

crystalline material which he named biotin, and which contained most of the yeast-stimulating properties of bios IIb. This was subsequently shown to be biotin methyl ester. In 1939 it was shown that coenzyme R, a growth factor for the legume root nodule bacterium, *Rhizobium trifolii*, is identical with biotin. Boas, in 1927, described a specific dermatitis produced in rats on a diet containing considerable amounts of raw dried egg-white, and showed also that a variety of foodstuffs contain a " protective factor X " which cures and prevents the appearance of the syndrome. György investigated Boas's protective factor X, and called it vitamin H (from the German, *Haut*, meaning skin). Vitamin H was identified with biotin in 1940 by György, du Vigneaud, and others.

Vitamin B_W, it has been suggested, is identical with biotin, and vitamin B_W is related to, if not identical with, factor W or vitamin W. Thus the following may be regarded, with varying degrees of certainty, as synonyms of biotin :—

Protective factor X (Boas)
Bios IIb (Kögl)
Coenzyme R (Allison *et al.*)
Vitamin H (György)
Anti-egg white injury factor
Vitamin B_W (Kringstad and Lunde) (?)
Factor W (?)
Vitamin W (?) (Elvehjem's rat growth factor)

To the above list of synonyms must be added the chemical name of biotin, 2′-keto-3 : 4-imidazolido-2-tetrahydrothiophene-*n*-valeric acid, for biotin has been isolated and synthesised. The isolation (of the methyl ester) was accomplished in 1940 by du Vigneaud, György, *et al.* Early in 1942 five possible structures were postulated for biotin, the empirical formula having been established as $C_{10}H_{16}O_3N_2S$. Eventually it was demonstrated that the structure is such as is represented by the following formula :—



B

The proof of this structure was long and complex. The details have been described by Melville (" Vitamins and Hormones," vol. II, p. 29, Academic Press Inc., New York, 1944).

Biotin methyl ester is the compound generally produced and which is coming into use clinically. This compound is soluble in ethyl alcohol, acetone and chloroform, but almost insoluble in water and ether. It has a melting point of about 167° C. and occurs in long acicular crystals. Free biotin occurs in a similar form, but has a melting point of about 232° C. It is soluble in dilute alkali or in hot water, sparingly soluble in dilute acid or cold water and insoluble in organic solvents.

Biotin appears to be a constituent of every living cell, but it is generally present only in extremely minute amounts. Indeed, liver, one of the richest sources, contains less than one part in a million. Other fairly rich sources are kidney, pancreas, milk and yeast.

Biotin is an essential constituent of the diet of most animals, including man. The requirements are so minute, however, that deficiency symptoms are not easy to produce, although a deficient diet during treatment with sulphaguanidine or succinylsulphathiazole may result in a deficiency. Before the introduction of these sulphonamides it was necessary to give raw egg-white in order to produce a deficiency. This recalls the work of Boas, which has since been explained by the fact that raw egg-white contains a protein, avidin, which combines with biotin and inactivates it. Cooked egg-white does not have this anti-biotin activity.

During 1942 four humans were given a diet in which 30 per cent. of the calorie requirement was provided by 30 per cent. of desiccated raw egg-white. They developed a desquamative dermatitis and pronounced pallor of the skin and mucous membranes. Other symptoms were muscular pains, lassitude, and mental depression. A concentrate providing the equivalent of 150 to 300 γ (0·15 mg. to 0·3 mg.) of biotin daily rapidly cured these patients.

Malignant tumours appear to contain a higher concentration of biotin than normal tissues, and attempts have been made to control malignancy by giving avidin or egg-white, but results were negative.

It would appear from the foregoing that avidin is entirely

a toxic protein which could be completely excluded from the body with advantage. That this is not necessarily so is suggested in a report from the U.S.A. The hypothesis is put forward that the antibacterial enzyme of lachrymal secretion, lysozyme, is a biotin-avidin complex in which the avidin is an essential carrier-protein. Lysozyme, it is suggested, acts by lysing (in this case "depolymerising") a mucoid constituent of certain bacterial membranes. It is further suggested that biotin may combine with other protein-carriers to form the hyaluronidases, or spreading-factors, which also act by depolymerising glycuronic acid compounds. Among the hyaluronidases are those of spermatozoa (which dissolve the cumulus cells and corona radiata of the ovum as a preliminary to the fusion of male and female gametes) and those of bee venom, leeches and invasive bacteria. The hyaluronidases in many instances may also be lecithinases, the mechanism of action of which has been described in the *Transactions of the Royal Society of Tropical Medicine and Hygiene*, July 1943, p. 1 (a general discussion) and in the *Lancet*, April 14, 1945, p. 457 (lecithinase and hyaluronidase of *Clostridium welchii* toxin).

The chemistry of the action of biotin in the body is not known, but there is one suggestion that it may act as a coenzyme in CO_2 transfer by an opening and closing of the urea ring in its molecule.

The available evidence indicates that the clinical indications for the use of biotin are desquamative and perhaps seborrhœic dermatoses, especially if accompanied by muscular pains, lassitude and mental depression. Such symptoms are most likely to appear in patients with a dietary idiosyncrasy which includes the ingestion of large amounts of raw eggs or who are undergoing treatment with sulphaguanidine or succinylsulphathiazole for intestinal infections such as bacillary dysentery or ulcerative colitis.

The usual amount of biotin excreted in the urine is 0·029 mg. to 0·062 mg. daily. Therapeutic doses of biotin may be between 0·025 mg. and 0·3 mg. daily, and such doses may be given subcutaneously or intramuscularly. It may be more convenient to give the larger doses intravenously.

Much more clinical investigation is necessary before all the

indications for biotin therapy are established. The high degree of biological activity of biotin seems to indicate that its uses will prove to be extensive as a pharmacological agent, although its use simply as a vitamin may be restricted in view of the rarity of deficiency states.

The remainder of the factors in this group are substances which are related functionally in that they are antianæmic factors, and related also in that there is possibly some unelucidated chemical connection between them. The factors are vitamin M, folic acid and xanthopterin. Two other substances, α-pyracin and β-pyracin, should perhaps also be included with these antianæmic factors, but there is little available information on their nature or function.

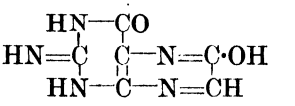
Vitamin M

The existence of vitamin M is fairly well established, but so far it has been shown to be essential only for monkeys of the genus *Macaca*, the macaques, of which the most familiar is the rhesus monkey (*Macaca mulatta*), which has come into particular prominence in connection with the Rh factor. (The Rh factor, it will be recalled, is responsible for the development of erythroblastosis fœtalis.) It has been suggested and denied that vitamin M and folic acid are identical. On the whole it seems reasonable to suppose that they are not identical, but that there is possibly some chemical relationship, and vitamin M is probably contained in certain concentrates of folic acid.

The symptoms of vitamin M deficiency in macaques, and possibly other primates, are diarrhœa, cytopenia, anorexia, progressive anæmia (sometimes macrocytic but usually normocytic), leucopenia, buccal lesions, lowered resistance to bacterial infection (especially dysentery), ulcerative colitis and œdema. This syndrome constitutes monkey pellagra, and it is possible that a similar condition may arise in man. A deficiency should be particularly watched for during treatment with sulphaguanidine or succinylsulphathiazole, and in this connection, and in view of the possible relationship between vitamin M and folic acid, it is of interest to note that it has been shown that the activity of these two substances is of a similar order. Xanthopterin is partly effective in correcting monkey pellagra, and it has been definitely attributed with hæmopoietic pro-

perties. Further, folic acid is thought to be a nucleoprotein with a molecular weight of about 500. (See note at end of chapter.)

Xanthopterin has the structure shown below, and it will be seen that it is closely related to the purines and can perhaps form compounds analogous to the purine nucleotides.

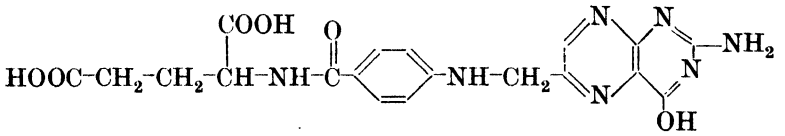


At the time of writing neither vitamin M nor xanthopterin is available commercially for clinical use.

Supplementary Note on Folic Acid

Folic acid was isolated in 1941 (*J. Amer. Chem. Soc.*, **63**, 2284) and eventually identified with Hogan *et al's.* vitamin B_c [isolated in 1943 (*Science*, April 30, 1943, p. 404)]. Some account of the chemistry of folic acid was given in *J. Amer. Chem. Soc.*, 1944, **66** : 271, and its synthesis was announced but not described in 1945 [*Science*, Aug. 31, 1945, p. 227, and *J. Indust. and Eng. Chem.* (News Edit.), Sept. 10, 1945, p. 1561]. The structure and method of synthesis of folic acid were not announced until 1946 (*Science*, May 31, 1946, p. 667).

Folic acid is N-[4-{[(2-amino-4-hydroxy-6-pteridyl)methyl]-amino}benzoyl]glutamic acid and its structure is represented by the formula :—



It is a yellow substance insoluble in water, active by mouth and by injection. Folic acid has been issued in the U.S.A. under the proprietary name " Folvite." A solution in oil in ampoules is issued for parenteral administration. Folic acid is not available commercially in Great Britain at the time of writing, but its general availability is expected in the near future.

Interest in folic acid is centred around its use in the treatment of macrocytic anæmias, particularly pernicious anæmia

and the anæmia of sprue. Over comparatively short periods folic acid has been shown to be remarkably effective in these macrocytic anæmias. In the early stages it brings about a prompt remission, blood count, cell size and hæmoglobin concentration rapidly returning to normal after a prompt and marked reticulocytosis. The effect of folic acid in maintenance of pernicious anæmia patients over a period remains to be established.

The doses of folic acid necessary to bring about a remission in pernicious anæmia and to cure the macrocytic anæmia of sprue has not been determined, but it appears to be of the order of 10 mg. to 30 mg. daily, the dose in pernicious anæmia perhaps being somewhat lower than in the anæmia of sprue.

The information so far available indicates that the same dose is as effective orally as parenterally. This, together with the fact that on a weight basis the dose of folic acid is considerably larger than that of the liver principle, indicates that folic acid is not identical with the hæmopoietic principle of liver. It is probable, however, that folic acid or a derivative of it is a constituent of the molecule of the liver principle, and the production and synthesis of folic acid will doubtless do much to promote the investigation of the chemistry of liver and its hæmopoietic principle(s).

Berry and Spies have given a comprehensive review of folic acid and its possible relationship to vitamins M and B_c and the growth factors for *Lactobacillus casei* and *Streptococcus lactis R*, and have suggested a possible relationship between these factors and the metabolism of thymine (methyluracil) which has been shown to have hæmopoietic activity (*Blood*, vol. I, no. 4, July 1946, p. 271).

CHAPTER XXVI

VITAMIN C

IN general outline the story of vitamin C and of scurvy—the state produced by avitaminosis C—is well known. Scurvy has been recognised from classical times, but a decrease in its incidence began in Europe early in the seventeenth century, and the condition is now rare and is only likely to be encountered under exceptional circumstances. The onset of the decrease in incidence coincided with the introduction of potatoes into Europe. Potatoes cannot be regarded as being particularly rich in vitamin C, but the quantities in which they are eaten are such that they provide perhaps half the average person's intake. There has perhaps been an increase during the last two centuries in the amounts of other fresh vegetables and fruits eaten; both these factors must be regarded as being mainly responsible for the present relative rarity of the disease.

The special value of lemon juice in preventing and treating scurvy was recognised as early as 1593 by Sir Richard Hawkins. Lind (in 1757) and Captain Cook (in 1772 and 1775) were aware of the value of lemons, oranges and fresh fruit and vegetables generally in the prevention of scurvy. In 1804 provision was made for providing all ratings in the British Navy with a daily ration of lemon juice. At a later period, during a period of shortage of lemon juice, "lime juice" (or perhaps more correctly lime-juice cordial) was supplied as a substitute. This is of little or no use for the purpose, but by some bureaucratic mischance the substitute became more or less permanent. Lime juice as an antiscorbutic is comparable with some of the other "antiscorbutics" which have been suggested in the past, such as vinegar, cider, and vitriol.

It is remarkable how near has been the discovery of the cause of scurvy on many occasions during the course of five or six centuries. It was suspected that scurvy was due to faulty or improperly salted meat or to an infection, effects

which, it was supposed, were counteracted by fresh fruit and vegetables. The conception of scurvy as a deficiency disease did not arise, however, until Funk postulated an antiscorbutic vitamin in 1912. Thereafter attempts were made to isolate the vitamin. Zilva *et al.* produced a concentrate between 1924 and 1929 which was active in guinea-pigs in 1 mg. to 2 mg. doses given daily.

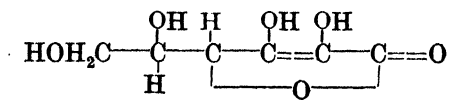
Szent-Györgyi isolated vitamin C from cabbage and from adrenal glands in 1928 without realising that it was the vitamin. He first called the substance " ignosic acid " from its relationship to a sugar which he called ignose. The name " ignose " was objected to, so he suggested " godnose " as an alternative. This too was not considered acceptable ! so he finally called it hexuronic acid. Waugh and King isolated vitamin C from lemon juice and from orange juice in 1932 and identified it with Szent-Györgyi's hexuronic acid.

The activity of synthetic vitamin C has been questioned and there has been a suggestion that the synthetic vitamin lacks some of the antiscorbutic properties of an amount of lemon juice containing an equal weight of the natural vitamin. The doubt is now rarely expressed, but there remains the possibility that vitamin P may play some part in controlling the hæmorrhagic manifestations of scurvy. The point is now not of vital importance because there is an increasing tendency not to rely entirely on pure vitamins or vitamin concentrates for the cure of any specific disease, but to improve the patient's diet generally and thus to provide any necessary secondary factors.

Chemistry of Vitamin C

Vitamin C is described in the B.P. (First Addendum) under the name ascorbic acid. In the U.S.A. it was known officially for a time as cevitamic acid, but this name is now rarely used and the name ascorbic acid is officially recognised.

Ascorbic acid is represented by the structural formula :—



This structure is described by the chemical name 3-keto-*l*-gulofurano-lactone (enol form). It is a colourless crystalline

substance with a melting point of 192° C, freely soluble in water and slightly soluble in alcohol. Ascorbic acid is fairly stable if protected from ultra-violet light and from oxygen in the presence of moisture. Iron, and more particularly copper, catalyses the oxidation of ascorbic acid, even when present only in minute traces. Heat accelerates the oxidation of ascorbic acid, but in the cooking of food there is a still greater loss from solution in the cooking water, which is generally discarded. A further loss in food is caused by the action of ascorbic acid oxidase, which is present in fruits and vegetables but is inactivated by heat during cooking. Ascorbic acid is more stable in acid solution than in the presence of alkalis, and fruit acids are therefore to some extent protective.

Oxidation of ascorbic acid may be reversible or irreversible. The product of reversible oxidation is dehydroascorbic acid, which retains the antiscorbutic properties of the original ascorbic acid. Methylene blue and indophenol dyes, which are used in the chemical estimation of ascorbic acid, are decolorised in the process of oxidising ascorbic acid. The vitamin is converted into dehydroascorbic acid. Thus chemical estimation gives an accurate indication of the potency of pure ascorbic acid, but it is of no value in estimating the total vitamin activity if appreciable amounts of dehydroascorbic acid are also present.

Units and Potency of Vitamin C

Before a chemical method of estimating ascorbic acid had been discovered the vitamin was estimated biologically in terms of its curative antiscorbutic activity in guinea pigs. The criterion of activity was either its effectiveness in preventing loss of weight or by its protective action on the teeth. In 1931 the League of Nations Conference on Vitamin Standards adopted fresh lemon juice as the standard preparation, and the vitamin activity of 0·1 c.c. of this was defined as an international unit. This unit is actually the potency of 0·05 mg. of pure *l*-ascorbic acid, so that 1 mg. has an activity of 20 international units. The unit is now rarely used, however, in stating doses of the vitamin. The ready availability of the pure substance renders the unit unnecessary, and doses are best expressed in terms of weight.

Ascorbic acid is estimated chemically by means of the dye 2 : 6-dichlorophenolindophenol. Ascorbic acid reduces the dye to a colourless form and is itself oxidised to dehydroascorbic acid.

Silver nitrate is a useful indicator of ascorbic acid in tissues, forming black particles with the vitamin *in situ*.

Mode of Action of Vitamin C

Vitamin C is almost if not entirely concerned with protein metabolism, particularly with the anabolic or constructive aspect of metabolism. Undoubtedly its powerful reducing properties are of the first importance, but precise details of its action cannot be given until the mechanisms of protein chemistry are known in much more detail than they are at present.

Szent-Györgyi has suggested that ascorbic acid takes part in a respiratory system, the mechanism of which may be represented as follows :—

$$\text{Ascorbic acid} + O_2 \xrightarrow[\text{hexonidase}]{\text{Cu ions}} \text{dehydroascorbic acid} + H_2O_2 \quad . \quad (1)$$

$$\text{Flavone} + H_2O_2 \xrightarrow[\text{peroxidase}]{} \text{oxidised flavone} + H_2O \quad . \quad (2)$$

$$\text{Flavone-oxide} + \text{ascorbic acid} \longrightarrow \text{dehydroascorbic acid} + \text{flavone} \quad . \quad (3)$$

$$\text{Dehydroascorbic acid} + \text{glutathione} \longrightarrow \text{ascorbic acid} + \text{oxidised glutathione} \quad . \quad (4)$$

$$\text{Oxidised glutathione} + \text{glucose phosphate} \longrightarrow \text{glutathione} + CO_2 + H_2O \quad . \quad (5)$$

In the absence of a flavone (for example, vitamin P) the reactions (2) and (3) may be replaced with :—

$$H_2O_2 \xrightarrow[\text{catalase}]{} H_2O + \tfrac{1}{2}O_2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

A second interesting suggestion which may give some indication of the chemical processes in the body in which vitamin C is concerned was made by Evans and Eames (*Med. Press and Circ.*, March 22, 1944, p. 184). " Glucose must first be phosphorylated before it can reach the cell. The electron transfers which effect the stage-by-stage metabolism require alternating ferric-ferrous (Fe^{+++} Fe^{++}) reactions for which

vitamin C is the essential catalyst. One of the by-products of this combined reaction is NaH_2PO_4, which in its turn produces the gastric HCl by the formula: $NaH_2PO_4 + NaCl = N_2HPO_4 + HCl$. It is well known that achlorhydria is common with both hypochromic anæmia and diabetes, and in the case of the former Davidson and Fullerton have shown that it develops at the same time as the result of a common cause, that being a nutritional deficiency. Our findings strongly suggest that this deficiency is vitamin C."

The clearest conception of the mode of action is perhaps obtainable from a consideration of the role it plays in various body-tissues. There is little doubt that the vitamin is essential for all body tissues, but the nature of its action varies to some extent according to the structure and function of the particular tissue under consideration.

Function in Connective Tissue

The principal constituent of connective tissue is collagen. During growth, in the normal process of replacement and in the repair and healing of wounds and burns, liquid collagen (or procollagen) is produced and flows into the intercellular spaces where it is required to form the cementing substance uniting the individual cells into firm tissue. One of the essential factors for converting the fluid procollagen into the firm collagen is ascorbic acid. If there is a deficiency, the conversion is incomplete and the tissue formed is weak and unresistant to stress. Bearing this in mind, the principal symptoms of scurvy are readily explainable and their apparent diversity is eliminated, a unified picture becoming possible.

The gingival hæmorrhage is a result of imperfect renewal of the tissues of blood vessels and of the mucous membrane. Wounds heal slowly and the new tissue is weak and readily breaks down under stress. It has even been noted that wounds which have healed during deficiency states may break down spontaneously during subsequent periods of deficiency. Similarly there may be a breaking down of callus of old united fractures. With the general connective tissue, the organic matrix of bone may be included, and this too is defective in the absence of adequate amounts of ascorbic acid. Thus the bones are likely to be friable and their structure will be

such that normal deposition of calcium is impossible, so that bones are lacking in elasticity, strength and rigidity.

Function in Bone Formation

The relationship of ascorbic acid to bone may be considered in greater detail.

It has been suggested that ascorbic acid may have three distinct rôles in bone regeneration (*Lancet*, Dec. 5, 1942, p. 661). These rôles are :—

1. To promote the activity of fibroblast cells and their differentiation into osteoblasts.
2. To promote attachment to each other of the polypeptide chains of the collagen fibres of bone matrix.
3. To promote phosphatase activity and so the precipitation of bone salts.

Ascorbic acid is thus necessary at all stages of bone growth, from the formation of the bone-forming cells, through the stage of formation of the organic matrix to the final calcification of this matrix to produce firm strong bones. Similar rôles may be assumed in the formation of teeth. It will be obvious that an adequate intake of ascorbic acid is especially important during growth and for the secure union of bone fractures.

Function in the Epithelium

Skin lesions due to hypovitaminosis-C include a marked dryness of the skin, noticeable fragmentation of hairs and small hyperkeratotic papules, first on the buttocks and posterior aspect of the calves and extending in more severe deficiency states to other areas. These lesions may be confused with those of hypovitaminosis-A, but in this latter condition the fragmentation of the hairs is absent. Again, in hypovitaminosis-A the hairs are commonly coiled up like watch-springs in dried perifollicular serous exudate.

Rosenberg (*Arch. Dermat. and Syphilol.*, 1938, **37**, 1010) has attributed certain cases of urticaria to hypovitaminosis-C, but this is more properly considered under the heading of the function of the vitamin in the metabolism of serum proteins and antitoxins which follows.

Function in Serum Protein Metabolism

The function of ascorbic acid in the protein metabolism of the body tissues so far considered is reasonably well established. The suggestions under this heading are somewhat more controversial, but there appears to be considerable and growing evidence of their essential validity.

As has already been mentioned, urticaria has been attributed to ascorbic acid deficiency, and in addition to this there have been attempts to correlate undue susceptibility to infection to the same cause. Anaphylactic shock can be prevented or minimised in animals by the administration of ascorbic acid, and hypersensitivity to a second injection of horse serum is greater in ascorbic-acid-deficient animals than in normal animals. It has been demonstrated that smooth muscle responsiveness to non-specific stimulation in guinea-pigs lacking in vitamin C is subnormal. Antibody response to specific antigenic stimulation is adversely affected by deficiency of the vitamin, so that the two basic factors (smooth muscle contractility and antibody production) are directly influenced. Inconclusive results have been obtained in asthma and hay-fever treated with vitamin C, and attempts to correlate vitamin C intake with serum complement level have also not shown uniformity or invariable agreement.

Although detailed and final conclusions are not justifiable, it is reasonable to assume that ascorbic acid is an essential factor for the production of antitoxins and of antibodies generally. Its rôle in the metabolism of the structural proteins of the body proteins, in connective tissue, bones, blood vessels etc., is probably intimately connected with the process of uniting molecules of polypeptides or the smaller proteins into larger, less soluble and firmer molecules, as, for example, in the conversion of procollagen into collagen. A similar rôle may be postulated for the vitamin in the formation (autosynthesis) of the molecules of antibodies. This process is being investigated intensively, but it is not yet sufficiently well understood to suggest just how the vitamin acts in this connection.

Ascorbic acid may play a similar part in the formation of red blood cells. Israels (*Lancet*, Feb. 6, 1943, p. 170) has suggested that a deficiency of ascorbic acid slows up the whole hæmopoietic process, a suggestion which is in agreement with

the views of Mettier and Chew (*Journ. Exp. Med.*, 1932, **55**, 971) and of Parsons (*Lancet*, 1938, **1**, 123). The subject appears to call for further investigation, however, for it has not been explained why the anæmia may be microcytic, normocytic or macrocytic.

Ascorbic acid may also be concerned in hæmoglobin formation, for some hypochromic anæmias which do not respond to iron have been reported to respond to the vitamin.

As an example of the action of vitamin C on the metabolism of an amino acid, which incidentally may provide a clue to its mode of action, its effect on tyrosine may be mentioned. If tyrosine is given in excess during ascorbic acid deficiency it is incompletely oxidised and is excreted as homogentisic acid. If adequate ascorbic acid is given at the same time as the tyrosine the oxidation of the latter becomes normal and the urine becomes normal in colour, as homogentisic acid is not present.

Function in Endocrine Metabolism

Guinea-pigs and primates, including man, are unable to synthesise ascorbic acid. It is therefore an essential dietary factor for them and thus a true vitamin. All other animals and plants synthesise ascorbic acid, and for them it is therefore a hormone. In view of this it is to be expected that ascorbic acid will prove to have a marked effect on the endocrine organs and on the hormones generally. It might be expected further that it will prove to have a particularly marked effect on those hormones which are of a polypeptide or protein nature. It is curious that although ascorbic acid has an effect on these glands or on the hormones secreted by them, its effects are not so marked as might be expected. It is remarkable, however, that two steroid-secreting glands—the suprarenal cortex and the corpus luteum—are particularly rich in vitamin C.

Vitamin C is said to potentiate the activity of the gonadotropic hormones. Vitamin and hormone given collaterally to rats produce a greater enlargement of the genitalia in male and female rats than either substance given alone. Adrenaline, a simple amino acid derivative, is probably protected from oxidation by vitamin C, and the vitamin also appears to exert a protective action on the steroid hormones of the adrenal

cortex. The relationship of vitamin C to the thyroid is obscure, but it does appear to be of some value in counteracting some of the adverse effects of hyperthyroidism, such as headache, nervousness, tremor, and sweating. The metabolic rate is unaffected, but the creatinuria is rapidly diminished.

There is little doubt that vitamin C is intimately concerned with the functioning of the corpus luteum. A low level of ascorbic acid excretion has been observed regularly at the time of ovulation (*Ind. Med. Gaz.*, Feb. 1940, p. 91), and the vitamin content of the corpus luteum is apparently directly proportional to its progestin content. Further, Israel and Meranze have stated that vitamin C has a progestin-like effect on the rabbit endometrium (*Endocrinol.*, 1941, **29**, 210) and Bourne (*Austral. Journ. Exp. Biol. and Med. Sci.*, 1935, **13**, 113) believes that vitamin C is concerned non-specifically with the production of the corpus luteum. This latter may well be a specific example of vitamin C as an essential factor in protein anabolism.

Ox suprarenals were one of the first sources from which vitamin C was isolated. It is found in both the medulla and the cortex of the glands, and it is thought that it may serve to stabilise the hormones secreted by these tissues—adrenaline and the 17-ketosteroids. The vitamin has an inhibitory action on the formation of pigment both from adrenaline and from 3 : 4-dihydroxyphenylalanine (" dopa "), so that its action in reducing the epithelial pigmentation in Addison's disease is understandable.

Clinical Uses of Vitamin C

On the rare occasions on which scurvy in adults is encountered, dramatic results follow the administration of vitamin C in adequate doses. In addition, the diet should be enriched particularly by the addition of fruits, fruit juices and fresh vegetables. Efficient oral hygiene should be maintained in order to prevent infection, and local lesions should receive symptomatic treatment.

Infantile scurvy (Barlow's disease) is likely to be encountered in practice, and treatment should be on similar lines, with the addition of light splints for the legs if these are required.

The nature and even the existence of " subclinical scurvy " or the " prescorbutic state " is frequently debated upon

Diagnosis of a prescorbutic state simply on the evidence of a low level of excretion of ascorbic acid certainly does not appear to be justified, for many individuals can be found who have low urinary ascorbic acid levels but satisfactory plasma levels and no definite symptoms attributable to deficiency of the vitamin. In spite of these observations, however, it is probable that hypovitaminosis-C is not uncommon, though it is not easy to decide which conditions are classifiable as clinical and which as subclinical. Diagnosis of deficiency must therefore be made on a basis of three considerations :—

1. The probable or (if possible) the actual vitamin content of the diet
2. The symptoms attributable to deficiency
3. The excretion of ascorbic acid

Deficient diets are not unlikely as a result of limited availability of fruit and vegetables through actual shortages, through poverty, through ignorance as to what constitutes a satisfactory diet or through idiosyncrasies in regard to food.

The minimum intake of vitamin C for an adult which is not normally likely to give rise to definite deficiency symptoms is probably of the order of 25 mg. to 30 mg. daily. A more satisfactory intake is 50 mg. to 75 mg. daily, increased in women to 75 mg. to 100 mg. daily during pregnancy and up to 150 mg. daily during lactation. Children should receive the following amounts :—

1 to 3 years	30 mg.
4 to 6 years	50 mg.
10 to 12 years	75 mg.
16 to 20 years	100 mg.

A seasonal variation in vitamin C nutritional level has been observed, reaching its lowest point in the spring when fresh vegetables are still scarce and the body has been depleted during the winter months of shortage of fresh vegetables. Conditions which have been attributed to this deficiency in the spring are increased susceptibility to infection and to fatigue, acute gingivo-stomatitis, chronic gingivitis. Anæmia of a microcytic hypochromic type may be another result, and this may lead to menstrual disturbances. Alternatively the menstrual dis-

turbances may be due to inadequate corpus luteum function. All of these conditions in which, as has already been stated, the clinical or subclinical status is debatable, may be relieved following the administration of vitamin C or by in increase in the amount of foods containing vitamin C.

Bicknell and Prescott ("The Vitamins in Medicine", Heinemann, 1942) give a comprehensive list of conditions in which a deficiency has been observed. No indication is given of those conditions which are due to the deficiency and those in which the deficiency arises as a consequence of the primary condition. but many of them are considered in the text, and the indication is, of course, given there. The conditions which are certainly, or which may be, a direct outcome of hypovitaminosis-C include hypochromic anæmia (iron resistant), leukæmia, purpura, achlorhydria, cataract, gingivitis, gingival hæmorrhage, and delayed healing of wounds and fractures. To these must be added scurvy and Barlow's disease (infantile scurvy). In all these conditions the administration of vitamin C constitutes the principal treatment, although collateral symptomatic treatment is also necessary in most instances.

A second group of diseases extracted from Bicknell and Prescott's list in which the administration of vitamin C forms an important but not the principal part of treatment may be given here :—

Addison's disease	Melæna
Asthma	Nutritional microcytic anæmia
Cœliac disease	Peptic ulcer
Coryza	Senile eye changes
Eczema	Sprue
Exfoliative dermatitis	Steatorrhœa
Hæmatemesis	Ulcerative colitis
Herpes zoster	Urticaria

The remaining conditions in which a deficiency of vitamin C has been noted consist of pyrexial states of bacterial infection, a group of metabolic and endocrine disturbances, of psychological disorders, and, finally, a group of dermatoses.

It is not suggested that vitamin C is a "cure-all", as might be inferred from the wide range of diseases mentioned, but on consideration it will be seen that all the conditions mentioned

concern some aspect of protein metabolism. This, as has been shown in the preceding section on the mode of action of vitamin C, brings all these diseases under this one heading. It is justifiable, therefore, to give vitamin C in any of the conditions named or in any of the conditions which may be included in the classes in the third group in which a coincidental hypovitaminosis-C has been observed.

Hitherto no mention has been made of the detoxicating properties of ascorbic acid. Antisyphilitic treatment with bismuth, arsenic or mercury may give rise to toxic reactions, and these may be prevented or minimised by giving the vitamin collaterally. Indeed, bismuth ascorbate has been suggested as a non-toxic antiluetic, but this compound of the vitamin is not generally available. Similarly, ferrous ascorbate has been used, partly by reason of the acceptability of iron in this form to the patient, but also by reason of a supposed increased absorbability of iron in this form. Ascorbic acid also counteracts the toxic effects of other heavy metals without impairing their therapeutic efficacy. It may be administered, therefore, in conjunction with gold compounds in arthritis and tuberculosis and with the mercurial diuretics. In large doses ascorbic acid has a diuretic activity which is said to be equal to that of digitalis.

Administration of Vitamin C

For prophylactic purposes and in most of its therapeutic indications vitamin C is administered orally. The vitamin is readily absorbed (from the small intestine) in almost all conditions. In achlorhydric patients, however, there may be appreciable decomposition in the stomach, and parenteral administration may be necessary.

Because of the protective effect of gastric hydrochloric acid on vitamin C, it is generally suggested that it should be given just before or during the course of a meal.

When given parenterally, vitamin C is injected intravenously or subcutaneously in the form of a solution of sodium ascorbate.

Estimation of Vitamin C Deficiency

Approximate estimations of vitamin C are easily carried out,

so that this is undertaken more frequently by the clinician than is the estimation of any other vitamin.

The most convenient method is based upon the decolorisation of 2 : 6-dichlorophenolindophenol. The reaction is not entirely specific for vitamin C, but valuable information can be obtained from its use in estimations of urinary content of the vitamin.

Dosage of Vitamin C

The pharmacopœial doses of vitamin C (ascorbic acid B.P.) are as follows :—

Prophylactic . . .	25 mg. to 50 mg. daily
Therapeutic . . .	100 mg. to 250 mg. daily

These doses are intended for adults. Infants are commonly given smaller doses prophylactically (of the order of 5 mg. to 15 mg. daily). It is especially important that artificially fed infants should receive the vitamin daily. Their need is relatively high by reason of their rapid growth, and artificial foods are practically devoid of the vitamin. A common practice is to give orange juice or tomato juice to provide the vitamin, but this may not always be readily available. Further, some infants are intolerant to these fruit juices. Tablets are available each containing 5 mg. of vitamin C and equivalent in terms of vitamin C to two teaspoonfuls of orange juice. Older children, adolescents, and adults may be given 25 mg. of vitamin C once or twice daily in prophylaxis.

In the treatment of actual deficiency states 25 mg. to 50 mg. daily may be sufficient for infants and young children, but 100 mg. should be regarded as the minimum therapeutic dose for adolescents and adults. The maximum pharmacopœial dose of 250 mg. daily is commonly exceeded, and as much as 1 grm. or even 2 grm. has been given in divided doses daily.

There is, for all practical purposes, no risk of producing adverse effects as a result of giving vitamin C in excessive doses. The greater risk is probably in giving less than is necessary to produce the optimum benefit, so that it is wise to err on the side of excess than the reverse. The larger doses may cause some degree of acidity in some patients, but this is evanescent, and may be reduced to some extent by giving such

doses during the course of meals rather than before meals, when the stomach is likely to be empty and at its highest level of acidity.

The minimum daily doses of vitamin C likely to produce satisfactory responses in most conditions in which it is indicated may be summarised as follows :—

	Children	Adults
Suspected deficiency states	50 mg.	100 mg.
Definite minor deficiency states	100 mg.	250 mg.
Severe deficiencies	150 to 200 mg.	300 to 500 mg.

The physician may frequently be inclined to give larger doses than these in individual cases and in special circumstances. He need not hesitate to do this should the occasion arise. It should be remembered, however, that the larger doses (say in excess of 300 mg. daily) need not be given over long periods. They are likely to produce saturation in about three to six days, and continued ingestion of amounts in excess of about 60 mg. to 75 mg. results in the excretion of considerable amounts without any additional benefit to the patient.

Special indications for particularly large doses over short periods are :—

(*a*) Pre-operatively and during convalescence : 1 grm. to 1·5 grm. on the two days before operation (may be given intravenously) and 100 mg. to 300 mg. daily (orally) during the following five to seven days. Rapid and satisfactory healing of the wound is promoted.

(*b*) During the pyrexial phases of severe bacterial infection : 0·5 grm. to 1 grm. daily during the fever (orally or intravenously) and 100 mg. to 200 mg. daily (orally) for a week or so after the temperature has become normal.

(*c*) To promote diuresis and mitigate the toxic effects of mercurial diuretics : 0·5 grm. given intravenously collaterally with the mercurial compound.

(*d*) During intensive treatment with compounds of arsenic, bismuth or gold : 200 mg. to 300 mg. intravenously with each dose of the metallic compound.

(*e*) In certain cases of anæmia resistant to iron or to liver extract: two or three daily doses of 300 mg. to 500 mg. may be given intravenously in addition to the specific treatment. Oral doses of 50 mg. to 75 mg. daily should be continued for a few weeks thereafter.

Other conditions calling for similar special treatment may suggest themselves to the physician.

Supplementary Note

A vitamin present in fruit juices, associated with vitamin C but not antiscorbutic, was postulated by von Euler in 1935. Little is known of this factor, called vitamin J by von Euler, except that he pointed out that it protects guinea-pigs from pneumonia. Vitamin J has also been called vitamin C_2, and there is a possibility that it may be identified with the "grass-juice factor" which was discovered by Elvehjem *et al.* in 1934 and shown to be necessary for the growth of rats and guinea-pigs (Kohler *et al.*, 1938).

CHAPTER XXVII

VITAMIN P

EXCESSIVE vascular and capillary permeability has been correlated with a dietary deficiency, and in 1935 Dam reported the existence of a "permeabilitäts vitamin". In the following year Szent-Györgyi *et al.* described a substance which increased capillary resistance in man, and named it citrin, or vitamin P. Citrin was found (1937) to be a mixture of the glycosides hesperidin and eriodictyol. On further investigation neither of these substances could be shown to possess vitamin P activity. Robeznieks, in 1938, re-examined citrin, and by means of chromatographic analysis showed that it contained a third unidentified flavanol glycoside similar to quercetin. The constituents of citrin identified up to this time were mostly insoluble, and their activity was attributed to their insolubility. A more soluble constituent was found in 1943 by Higby—hesperidin chalcone—which is said to have vitamin P activity. This substance readily reverts to hesperidin, but methylation produces a more stable substance. A related substance—quercetin rutinoside, or rutin—was reported by Griffith, Couch and Lindaur (1944) as increasing capillary resistance.

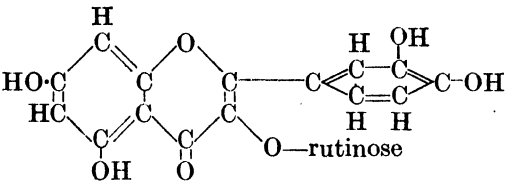
Rutin was first isolated in Manchester by Schunck in 1860. It is probable that it is the substance to which the various "concentrates" of "vitamin P" owe their activity. Rutin occurs widely in nature, in tobacco leaf, rue (hence the name "rutin"), tomato stems, yellow pansies, elder leaves and buckwheat. In the U.S.A. the production of rutin from the latter source has begun on a commercial scale.

Chemistry of "Vitamin P"

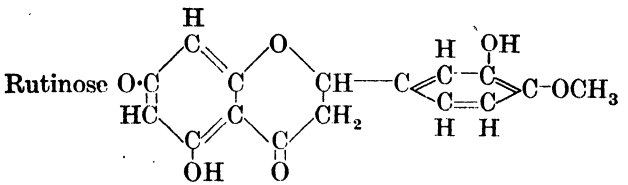
There seems to be little doubt that vitamin P will be proved to be a flavone glycoside. It has not been identified conclusively with any of the known glycosides, but the most active substance so far found is rutin or quercetin rutinoside (rutinose being the diglycoside, rhamnoglucose).

Rutin may be prepared by alcoholic percolation of green buckwheat. It is a bright yellow powder consisting of acicular crystals with a melting point of 192–196° C., soluble 13 in 100,000 in water at 20° C. It is soluble in alcohol, acetone, ethyl acetate and alkalis but insoluble in ether and chloroform.

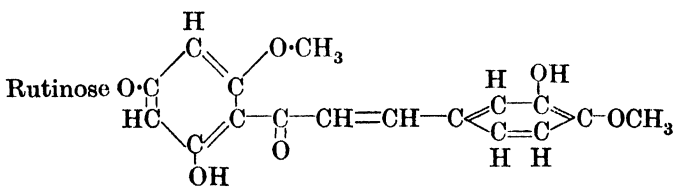
Rutin has the structure represented by the following formula :



It will be seen that this substance is closely related to hesperidin, which is :



Hesperidin methylchalcone is :



" Vitamin P " is reasonably stable, but activity is lost on boiling, and blackcurrant purée (a good source of the vitamin) slowly loses its activity even when stored in the dark in the absence of oxygen.

Mode of Action of Vitamin P

Szent-Györgyi has suggested that vitamin P may take part in a respiratory system in conjunction with vitamin C. (See p. 266.) The two vitamins commonly occur in close association in plants, and both vitamins appear to have a function in protein metabolism in animals. It is possible

that the vitamin has other functions, for it has been reported that hesperidin methyl chalcone reduces the toxicity of "Mapharsen" administered to rabbits and itself exerts a mild spirochæticidal action (*Science*, 1943, **98**, 245). This is of particular interest when considered in the light of a suggestion made in the *Journal of the American Medical Association*: "The rôle of glycosides in plants has been interpreted as a mechanism whereby the substances which have great physical [? physiological] activity are held inert until they are needed in the metabolism of the plant or in detoxifying poisonous substances, so that the latter mechanism maintains these substances for protecting the functional integrity of the vascular endothelial system in man (Scarborough, *Biochem. J.*, Sept. 1939, p. 1400) demonstrated in vascular purpura" (*Journ. Amer. Med. Assoc.*, Aug. 17, 1940, p. 519).

Szent-Györgyi's suggestion that vitamin P takes part in a respiratory system appears to be the only direct indication of its mode of action. The conditions in which it has been claimed that the vitamin is of value all indicate that it is concerned in protein metabolism. It has been suggested that it may contribute to the growth and hardness of bones and teeth (*Chem. Age*, Feb. 3, 1946, p. 214). There have been several claims that it protects the capillary endothelium against the toxic effects of various metals (arsenic, gold etc.) and against thiocyanates. Finally, vitamin P is necessary to correct the subcutaneous hæmorrhage characteristic of scurvy in which vitamin C alone is not completely effective.

It may be concluded therefore that vitamin P is necessary in laying down normal protein tissues, in ensuring the maintenance of the structure of these tissues and to protect them against various toxic substances.

Clinical Uses of Vitamin P

Lemon juice, orangé juice, prunes and blackcurrant purée are all good sources of vitamin P, and infants who are receiving one or more of these as a source of vitamin C are probably getting sufficient vitamin P to ensure the formation of normal bones and teeth. In those cases in which ascorbic acid alone is given it may be desirable to provide some additional source of vitamin P. Grapes, black or white, are not particularly

rich in vitamin C, but they are probably the richest dietary source of vitamin P.

In addition to its action on the capillary walls, it has been claimed that vitamin P accelerates both the beginning and termination of clotting time (*Schw. med. Woch.*, Feb. 15, 1941, p. 155, abstracted in *Journ. Amer. Med. Assoc.*, Aug. 8, 1941, p. 489), and it may be that both functions are important in controlling hæmorrhage. Vitamin P is indicated in the petechial hæmorrhages of scurvy, infantile scurvy, purpura hæmorrhagica and allergic states. It may be given to control hæmorrhage in hæmaturia and nephritis and the hæmorrhagic oozing sometimes noted after prostatic resection and lithotrity (*Canad. Med. Assoc. Journ.*, June 1940, p. 583).

Vitamin P is particularly recommended in all cases of hypertension, especially those which are being treated with thiocyanates. In hypertension there is an increased tendency to abnormal capillary fragility, which may be manifested as apoplexy or retinal hæmorrhage. Thiocyanates tend to increase this risk, but it is counteracted by vitamin P. The vitamin has been suggested for the reduction of hypertension, but there is no evidence that it has any effect on the blood pressure. Its effect is limited to reducing the risk of hæmorrhage. Vitamin P should be given collaterally with any toxic drug (such as compounds of arsenic or gold) which tend to increase capillary fragility and the consequent risk of hæmorrhage.

Dosage and Administration of Vitamin P

It seems probable that rutin will become generally available for clinical use in the comparatively near future and that it will be the drug of choice in all the indications for vitamin P therapy.

Rutin is administered orally, generally in doses of 20 mg. three times daily. In resistant cases or in severe deficiency states or during intensive dosage with thiocyanates, arsenic or gold preparations it may be necessary to give 40 mg. three times daily.

Untoward effects from unnecessarily large doses of vitamin P have not been reported.

PART III

CHAPTER XXVIII

HORMONES AND VITAMINS: DIFFERENCES AND SIMILARITIES

AS generally considered, hormones and vitamins are two distinct and sharply defined groups of substances. A closer acquaintance with the whole range of substances included in these groups, however, reveals that a sharp dividing-line between them can be laid down for one species of animal, but this will not necessarily be found to be valid for any other species. The outstanding example of this, of course, is ascorbic acid. Man, the other primates and guinea-pigs cannot themselves synthesise ascorbic acid. It is therefore a vitamin for them. Ascorbic acid is an essential for all other animals, but since they are able to synthesise it, it is for them a hormone.

Most, if not all, plants also need ascorbic acid. They are probably all able to synthesise it, so that for them it may be regarded as a hormone. Most of the *vitamins* required by animals are required by plants, but since plants are able to synthesise them, they are, for them, hormones.

This general statement holds good also for those substances synthesised by the intestinal bacteria of most mammals and the bacteria of the rumen in cattle which are hormones for the bacteria but are absorbed and utilised as vitamins by their hosts. In this group the vitamins of the B group and vitamin K are the outstanding examples.

It will be recalled that there is a possibility that certain members of the vitamin B group, notably xanthopterin, may prove to be constituents of the hæmopoietic principle of liver. This is regarded by some as being a hormone, so that it appears that here we have an intimate relationship of another kind between hormones and vitamins, the one actually entering into the structure of the other. An analogous relationship may exist between vitamin K and thrombin, which latter is

formed possibly by a chemical reaction between the vitamin and prothrombin. This is not fully understood, however, and the rôle of the vitamin may be purely enzymatic, in that it may not itself enter into the structure of the thrombin molecule, but only activate the conversion of the prothrombin into thrombin.

The œstrogens are produced in plants as well as in animals. For example, œstrogenic substances have been obtained from coal, and here it almost certainly had its origin in the living plants of the Carboniferous era. Some modern plants also yield œstrogenic substances, positive results having been reported with alder leaves and catkins, willow catkins, sprouted oats, and rhubarb leaves. Further, Butenandt has isolated "tocokinin" from palm kernels and identified it with œstradiol.

It has been shown that œstrogenic hormones accelerate the growth of plants and stimulate flower production, so that it seems reasonable to assume that œstrogens are hormones for plants as well as animals.

The chemical relationship and pharmacological similarity between adrenaline and ephedrine are well known, and it may well be that ephedrine will prove to be a variant of adrenaline, acting as a hormone in certain plants (ephedrine has been found in traces in the yew (*Taxus baccata*) as well as in the plants of the genus *Ephedra*, and may be found in other plants).

The Dividing Line

The dividing line between hormones and vitamins is thus to a considerable extent arbitrary, and depends largely on whether the organism under consideration happens to synthesise the particular substance it requires, or whether it is dependent upon dietary or other external sources of supply. Any one substance may be both a hormone and a vitamin, and in the broad view hormones and vitamins may be regarded as varieties of a larger group. Cawardias has pointed out the similarity between hormones and vitamins—"vitamins, the most important external regulators, a sort of external hormone". ("Constitutional Medicine and Endocrinology", vol. I, 1944).

The larger group suggested to include hormones and vitamins may be enlarged further to include alkaloids, glycosides, resins,

gums, essential oils, venoms, digestive and other enzymes, and the so-called " neurohormones ".

Sevag has suggested that " the enzymes and antigens not only belong to the same class of biocatalysts but that the enzymes and antigens represent a unity " (" Immuno-Catalysis ", 1945, Charles C. Thomas, Baltimore). Thus antigens, including bacterial toxins, may be included in the general class in which hormones and vitamins have been included. In this connection the formation of antihormones will be recalled as analogous to the formation of antitoxins.

All such substances, from the evolutionary point of view, perhaps had their origin as products of pathological conditions of the organism producing them. Subsequently, it may be supposed, the organism found some advantage in its own pathological product (hormones) or became dependent on the pathological product of some other organism (vitamin). In either instance the substance became normally necessary, increasing the metabolic efficiency or reproductive capacity, so increasing the powers of survival of the new variant and playing a part in the evolution of new types.

In each instance these substances do not enter into the structure of the organism producing them, but only contribute to its development or function. Some, however, of the substances in this large group have not been adopted as products of high metabolic importance and only fulfil a minor rôle if they are of any physiological significance. The gums and resins may be of this type and merely be metabolic waste products.

Some support is given to this suggestion of similarity in origin of this seemingly miscellaneous group of metabolic products by the fact that many of them, as well as a number of others, are, it has been suggested, built up from isoprene units (Heilbron, *Pharmaceut. Journ.*, Jan. 13, 1934, p. 31): Isoprene is :— $CH_2=\underset{\displaystyle CH_3}{\overset{|}{C}}-CH=CH_2$, and among the more important substances named as probably being built up from this fundamental unit are, terpenes and sesquiterpenes of essential oils, santonin, pine resin acids, flower pigments, carotenoids, chlorophyll, squalene (from elasmobranch fish-liver oils) and caoutchouc.

In contrast to the similarities between hormones and

vitamins, one difference between them may be mentioned, although it may be more apparent than real. Whereas the hormones are by definition " messengers ", being carried to their site of action as required, the vitamins generally appear to act *in situ* in combination with the more or less fixed intracellular proteins. " Bearer " proteins, or pherons, have not been described for any of the hormones although " receptors " have been postulated at the site of action of hormones and the effective functioning of these " receptors " may be an essential condition for the manifestation of hormone activity (*Brit. Med. Journ.*, Jan. 18, 1947, p. 79).

CHAPTER XXIX

HORMONES AND VITAMINS IN AN INTEGRATED VIEW OF METABOLISM

IT has been shown in the previous chapter that the similarities between hormones and vitamins are more remarkable than their differences. The enzymatic nature of the vitamins or of their biologically active compounds—the enzymes and coenzymes of the oxidation-reduction systems—is apparent, especially in the case of the principal vitamins of the B group. It is unnecessary to enlarge upon this, but it may be pointed out that the hormones are also essentially enzymatic in their mode of action.

The high physiological activity of both hormones and vitamins in minute amounts or in high dilution has been repeatedly emphasised as being characteristic of both groups of substances. This in itself is sufficient to suggest the possibility of their being enzymatic in nature and acting as organic- or biocatalysts. Such a conception of their function cannot be doubted in the case of the majority of the vitamins, and a consideration of the mode of action of the hormones will provide presumptive evidence in support of a similar conception of the function of the hormones.

The enzymatic nature of hormone action is particularly obvious in the case of insulin and of the thyroid hormone, and reference should be made to the sections dealing with the mode of action of these hormones in the relative chapters in Part I. The mode of action of the hormones of the pituitary gland is unknown, but their effect in stimulating the secretion of other hormones bears every evidence of being enzymatic in nature. The steroid hormones of the ovary, testes, and suprarenal cortex are perhaps not so obviously enzymatic, but under the influence of these hormones there is active building up and elaboration of tissue. This may be regarded as an enzyme-activated process, the converse, in some respects, of the enzymatic action of the thyroid and of insulin, the actions of

which are catabolic, whereas the gonadal steroid hormones act anabolically. At this point it is of interest to recall Shute's postulation of the antitryptic action of the œstrogenic hormone and of the opposing action of vitamin E (see chapter on vitamin E in Part II).

The production of antihormones, especially to chorionic gonadotropin, is essentially an enzymatic process if the postulates of Sevag are accepted ("Immuno-Catalysis", 1945). It is curious, however, that the body does not usually appear to elaborate "antivitamins" or, to express it in the more conventional way, the vitamins are for the most part non-antigenic, being unable to produce antibodies or "antitoxins". The nearest approaches to the formation of "antivitamins" appear to be indicated by the fact that cases have been reported in which a patient has developed a hypersensitivity, seemingly of an allergic nature, to vitamin B_1; that carp contain an anti-aneurine factor which is capable of rendering vitamin B_1 unavailable for silver foxes; and, finally, that raw egg-white contains an "antibiotin" factor—avidin—so that biotin-deficiency symptoms can be produced in a subject if excessive amounts of raw egg-white are administered.

Undoubtedly much evidence remains to be discovered, but enough is probably available to justify at least a tentative assumption to the effect that hormones and vitamins are essentially the active radicles in enzyme systems.

In Parts I and II of this book, in the sections dealing with the mode of action of the hormones and of the vitamins, some attempt has been made to emphasise the evidence available in each individual instance which tends to support this suggestion of the essential similarity of function of hormones and vitamins. On the basis of this evidence it is suggested that hormones and vitamins should be regarded as belonging physiologically to the same large group of metabolites and that they are really only divided into two separate groups by reason of the fortuitous circumstance of their having an endogenous or an exogenous origin.

This unified conception of the nature of hormones and vitamins, it is hoped, may assist in establishing a more coherent conception of these chemically heterogeneous groups of substances which will simplify the acquisition of a reasoned idea

of their nature and upon which can be built up a more easily remembered knowledge of their effective application in clinical and preventive medicine.

Such a conception is not to be regarded as complete or self-sufficient, but may be extended eventually so as to include other classes of originally pathological products which have, during the course of evolution, become adapted for specific physiological purposes or which may be in course of being so adapted. Such classes, as was indicated in the preceding chapter, include venoms, digestive and other enzymes, and the "neurohormones" in animals, and the alkaloids, glycosides, resins, gums and essential oils in plants.

Returning finally to a more specific viewpoint, the conception indicated herein may also contribute in some measure to the application of endocrinology and "vitaminology" to the practice of "constitutional" medicine as described by Cawadias in "Constitutional Medicine and Endocrinology", vol. I, Muller, 1944. In effect, Cawadias states that an integration of metabolism, endocrinologically and "vitaminologically", must form a basis for diagnosis and prescribing in the constitutional view of the patient which the physician must develop. To quote Cawadias (*ibid.*) ". . . endocrinology [and, he might have added, "vitaminology"] becomes thus a pathology of correlation and regulation. For this reason this branch of medicine demands a special form of synthetic intellect."

CHAPTER XXX

THE "TRACE ELEMENTS" AS HORMONAL AND ENZYMATIC PROSTHETIC GROUPS

THE distinction between the mineral elements required in "bulk" for body metabolism and those only required in "traces" is not clearly defined. In this they resemble certain of the vitamins; in particular, choline is regarded as a vitamin and as a "bulk" nutrient. Methionine, which is related to choline in function, has been considered as being a vitamin in some respects, but it is more definitely a "bulk" nutrient than is choline. Similarly with the essential mineral elements concerned in metabolism. As an example, calcium is certainly a "bulk" nutrient when considered as a constituent of bone, but it is perhaps more of the nature of a "trace" element in its function as a controller of nerve function. Although sodium is perhaps not a "bulk" constituent of the structure of the body, it is widely distributed and must be considered to be a bulk rather than a trace element.

Many mineral elements, however, play an essential rôle as constituents of enzymes or coenzymes, and for this reason merit some consideration in connection with the vitamins, which they resemble in certain of their functions.

Sheldon, in 1933, surveyed the "trace" elements as then understood and gave a comprehensive picture of their rôle in metabolic processes ("The Mineral Basis of Life", *Brit. Med. Journ.*, Jan. 13, 1934, p. 47). Many of his observations stand, and the following summary is based upon his account, modified and supplemented in accordance with more recent findings.

The Halogens

Iodine.—The thyroid hormone is perhaps the most important form in which iodine occurs in the body. Its effect in increasing the metabolic rate may be regarded as enzymatic in nature, and the chapter in Part I on this hormone should be studied. In no other hormone does an "inorganic" element assume such importance as does iodine in the thyroid hormone. In addition

to the high concentration of iodine in the thyroid, there is a notable concentration of this element in the tuber cinereum, just above the pituitary gland. The tuber cinereum probably controls the hypothalamus, and iodine is almost certainly concerned in this control. Thus iodine compounds are concerned in endocrine activity directly through the thyroid and indirectly through the higher control of the hypothalamus. Nothing is known of the chemistry or mechanism of action of iodine in the tuber cinereum; indeed, it is only inferred that it has such an action from the fact that it occurs in this situation in unusually high concentration.

Bromine.—The highest concentration of bromine occurs in the pituitary gland. Its function there is unknown, but it is significant that the blood bromine level varies during the menstrual cycle and that the blood level is reduced to half the normal value during manic-depressive psychosis. Further, the blood bromine level begins to fall at about forty-five years of age and is almost undetectable at seventy-five.

Chlorine.—In the form of sodium chloride, chlorine is to be regarded as a " bulk " constituent of the body. As hydrochloric acid it is an essential metabolite in enzymatic processes, but not as a biocatalyst in the usual sense of the term. Chlorine is therefore not regarded as a " trace " element in animal metabolism.

Fluorine.—In abnormal concentrations fluorine is an inhibitor of enzymatic processes, and there is no evidence that it acts " biocatalytically " in lower concentrations. In minute amounts (not exceeding 1 to 1·5 parts per million in the diet) fluorine is beneficial in that it combines with the mineral constituents of the teeth and renders them more resistant to decay, but the process is not a catalytic one.

Silicon

Silicon has been detected in the body (average blood level is 16 mg. of SiO_2 per 100 c.c.), but it is not known whether it has a specific function or not.

The Alkali Metals

Sodium.—Like chlorine, sodium is to be regarded as a " bulk " metabolite.

Potassium.—In spite of their close chemical similarity potassium and sodium are not interchangeable in metabolic processes, and potassium is essentially a "trace" element, although deficiencies must be rare. Potassium potentiates the muscular response to nervous stimulation. In this action it is antagonistic to calcium, which tends to diminish the response. For this reason potassium has been described as being "adrenalinogenic", but this may be a misnomer, in that there is little evidence to show that it is concerned in the synthesis of adrenaline. More probably its function is to sensitise muscle to the action of adrenaline. This may be a catalytic action.

Rubidium.—There is some scanty evidence that rubidium may be concerned in growth. It occurs in human milk, the amount in the liver is increased during the nursing period and the amount in liver has been stated to be abnormally high in infants dying from pyloric stenosis.

Lithium and *cæsium* have no known significance in animal metabolism.

The Alkaline Earth Metals

Beryllium.—There appears to be no record of beryllium occurring in animal tissues, but when given experimentally to animals it is capable of producing severe rickets. Thus it is in some way antagonistic to calcium.

Calcium.—Calcium is probably not a "trace" element in the ordinary sense of the term, although, in addition to its importance as a bulk element, it is important in that it reduces nerve sensitivity, and so is antagonistic to potassium in this respect.

Barium.—There have been a few isolated reports of minute traces of barium in the eyes of cattle. Nothing is known of its function in this connection, and it may well be that its occurrence is accidental.

Strontium.—Animal tissues readily take up strontium when it is available, but, as in the case of barium, there is no evidence that it is needed in any essential physiological process.

Magnesium.—There is little doubt that magnesium plays an essential enzymatic rôle in the body. It is certainly essential as an activator of the phosphatases and phosphorylases.

It will be recalled that the phosphatases are a group of enzymes, functioning at various degrees of pH, which are responsible for the conversion of inorganic phosphates into organic phosphates. The two most important of the actions in which magnesium plays a part are the phosphorylation of hexose (glucose or fructose) as the first step in carbohydrate oxidation and in the calcification of bone, involving a phosphatase. The manner in which magnesium acts in these processes does not appear to have been elucidated, but it is possible that it forms a prosthetic " group " in the coenzymes.

Copper

Much remains to be learned of the rôle of copper as a component of oxidation-reduction systems. It is widely distributed in both plant and animal tissues, and a number of copper-protein enzymes have been described. Most of these occur in plants, but some information is available on at least two animal enzymes—hæmocuprein and hepatocuprein.

Hæmocuprein is stated to account for all the copper in the blood. It is a protein compound containing about 0·34 per cent. of copper and having a molecular weight of about 35,000. The function of hæmocuprein is not known, but it is quite possible that it is concerned in the introduction of iron into the molecule of hæmoglobin, cytochrome, cytochrome oxidases, etc. Hepatocuprein may be a different protein or it may be a related substance with the copper in a different valency state.

The respiratory pigment of certain arthropods and molluscs is a copper-protein compound, hæmocyanin, and it is interesting to speculate upon the possibility of this being a chemical prototype of and the parent substance of hæmoglobin.

The distribution of copper in normal and pathological states suggests some important rôles for this metal in various metabolic processes. Normal growing tissues contain somewhat more copper than mature tissues, suggesting that it may be a growth factor. In contrast to this, it has been pointed out recently that there is a two-stage reduction in the copper and zinc content of skin as a result of the application of methylcholanthrene leading to the production of a carcinoma. Serum-copper levels, on the other hand, are stated to be raised in certain types of anæmia, in pregnancy, and perhaps in malignant

disease. The œstrogens appear to exert some effect in regulating serum-copper levels. This last point perhaps merits some consideration in conjunction with Shute's theory of the anti-œstrogenic properties of vitamin E (see page 183), for copper acts catalytically in inactivating vitamin E.

Manganese

Manganese is the metallic radicle in the respiratory pigment of one solitary species—the Lamellibranch, *Pinna squamosa*. In plants it seems to be essential for the incorporation of magnesium into the chlorophyll, though it does not carry out an analogous rôle in animals in aiding in the incorporation of iron into the hæmoglobin molecule.

It is curious that manganese acts in rats in some ways in a similar manner to vitamin E. A deficiency causes testicular degeneration and incomplete development and hyposecretion of the anterior pituitary. It may be assumed that manganese performs similar functions in humans, and McCarrison is of the opinion that the usual intake is " below the safety level ".

Cobalt and Nickel

There is no satisfactory evidence that cobalt is required by humans, but it may be capable of producing a true erythrocyte polycythæmia as it does in other mammals.

Nickel is said to be concentrated in the pancreas in humans, but this has not been confirmed, and no essential rôle has been suggested for this metal.

Zinc

Two zinc-protein compounds certainly exist, and are essential for humans. Insulin is one of these (see page 41), and the other is carbonic anhydrase, which catalyses the reaction

$$H_2CO_3 \rightleftharpoons CO_2 + H_2O.$$

The protein of carbonic anhydrase has not been identified, and it is not known what part the zinc plays in the action of the enzyme.

It is probable that other zinc-protein enzymes remain to be discovered.

Other metals are found in minute amounts in tissues, but

their physiological significance is unknown or in doubt. For example, silver has been found in the thyroid in appreciable amounts, and tin is said to occur in the muscle and more particularly in the mucous membrane of the tongue. Still other elements are known to occur in significant amounts in certain species of animals only. In these cases it appears that Nature has made isolated experiments in the utilisation of elements. Examples of this are : barium in the eyes of cattle, cadmium in the common scallop and vanadium in the respiratory pigment of certain ascidians.

GLOSSARY

PROPRIETARY names are indicated by the use of initial capital letters, and the manufacturers are indicated in abbreviated form, a key to the abbreviations bein ; included in the glossary. Manufacturers who issue products under generally accepted non-proprietary names are given under the name the manufacturer employs.

Completeness has not been aimed at in compiling this Glossary, but it is hoped that it will be of some value to physicians (synonyms and proprietary names particularly) and to pharmacists (medical and technical terms).

A. & H.—Allen & Hanburys, Ltd., Bethnal Green, London, E.2.
A.T. 10—dihydrotachysterol, *q.v.*
Abbott—Abbott Laboratories (Eng.), Ltd., Wadsworth Road, Perivale, Middlesex.
adenoma—benign enlargement or neoplasm.
Adexolin (Glaxo)—vitamins A & D in oily solution.
adrenaline—(epinephrine in the U.S.A.) (B.D.H., B.W., P.D. & Co., etc.).
Ambinon A (Organon)—thyrotropin with pituitary and chorionic gonadotropins.
Ambinon B (Organon)—thyrotropin with chorionic gonadotropin.
amenorrhœa—absence of menstruation.
amino acid—organic acid in which NH_2 (amino) replaces a hydrogen atom.
Amniotin (Squibb)—œstrone.
Androfort (Richter)—androsterone.
androgen—a hormone or synthetic substance with masculinising properties.
androsterone—an androgen, androsten-3-*cis*-ol-17-one.
aneurine—thiamine, vitamin B_1 (B.D.H.), 3-(4′-amino-2′-methyl-pyrimidyl-5′-methyl)-4-methyl-5-β-hydroxyethylthiazolium chloride (see also vitamin B_1).
p-anol—*p*-propenylphenol, possibly the active grouping, according to Linnell, of the synthetic œstrogens.
Antostab (Boots)—serum gonadotropin.
Antregone (Abbott)—chorionic gonadotropin.
Antuitrin G (P.D.)—anterior pituitary growth hormone.
Antuitrin S (P.D.)—chorionic gonadotropin.
aphanin—a carotenoid pigment, precursor of vitamin A.
arachidonic acid—a highly unsaturated fatty acid (*vide* " vitamin F ").
atresia—failure of rupture of a follicle or membrane, especially of ovarian follicles.
Avoleum (B.D.H.)—vitamin A, in solution in oil. (30,000 I.U. per gramme) and in capsules (each 4500 I.U.).
axerophthol—name suggested by Karrer for vitamin A.

B.D.H.—The British Drug Houses Ltd., Graham Street, City Road, London, N.1.
B-plex (Wyeth)—vitamin " B " compound.
B.W.—Burroughs Wellcome & Co., 12 Red Lion Square, London, W.C.1.
Bayer—Bayer Products, Ltd., Africa House, Kingsway, London, W.C.2.
Beflavit (Roche)—riboflavine.
Befortiss (Vit. Ltd.)—vitamin B_1.
Benadon (Roche)—vitamin B_6.
Benerva (Roche)—vitamin B_1.
benzestrol—2 : 4-di-(*p*-hydroxyphenyl)-3-ethylhexane, a synthetic œstrogen, formerly Octofollin (*q.v.*).
Benzo-Gyncestryl (Roussel)—œstradiol benzoate.
Benztrone (P. & B.)—œstradiol benzoate.
Berin (Glaxo)—vitamin B_1.
Betalin (Lilly)—vitamin B_1.
Betaxan (Bayer)—vitamin B_1.
bitterling—*Rhodeus amarus* (*Block*) (*q.v.*).
Boots—Boots Pure Drug Co. Ltd., Station Street, Nottingham.

calciferol—vitamin D_2 (B.W.).
carboxylase—aneurine pyrophosphate (cocarboxylase, *q.v.*) attached to a protein and forming the decarboxylating enzyme of the carbohydrate catabolic system.
carcinoma—malignant enlargement or neoplasm (cancer), *cf.* " adenoma."
carotene—one of the group of yellow plant pigments, some of which form vitamin A on hydrolysis.
Celin (Glaxo)—vitamin C.
cevitamic acid—obsolete American name for vitamin C.
chorionic—produced in or by the placenta, as chorionic gonadotropin (*q.v.*).
Ciba—Ciba, Ltd., The Laboratories, Horsham, Sussex.
climacteric—the period of endocrine readjustment associated with the menopause (*q.v.*).
Clinestrol (Glaxo)—stilbœstrol.
cocarboxylase—aneurine pyrophosphate, the coenzyme of carboxyglase (*q.v.*).
coenzyme—(*see* cozymase) a " secondary " enzyme, usually a hydrogen acceptor.
cophosphorylase—adenosine di-(or tri-) phosphoric acid, coenzyme of phosphorylase, the enzyme essential for the phosphorylation of hexose in the early stages of enzymatic carbohydrate decomposition.
Cortrophin (Organon)—adrenal cortical trophin (tropin ?) from anterior pituitary.
cozymase I—coenzyme I or diphosphopyridine nucleotide, a coenzyme or hydrogen acceptor for several of the dehydrogenating enzymes.
coenzyme II—the triphospho compound resembling coenzyme I.
creatine—methylguanidine acetic acid, a carrier substance for phosphoric acid in the carbohydrate metabolism system; its phosphate is phosphagen.
Crookes—Crookes Laboratories—Gorst Road, Park Royal, London, N.W.10.
cryptorchidism—non-decent of testes.
*cyclo*pentanoperhydrophenanthrene—also known as cythrene, basic saturated hydrocarbon of the steroids.
Cyren (Bayer)—stilbœstrol.

Cyren B (Bayer)—stilbœstrol dipropionate.
cythrene—abbreviated name for *cyclo*pentanoperhydrophenanthrene (*q.v.*).

Davitamon A (Organon)—vitamin A.
Davitamon B (Organon)—vitamin B_1.
Davitamin C (Organon)—vitamin C.
Davitamin E (Organon)—vitamin E.
Davitamon K (Organon)—vitamin K.
Dekadexolin (Glaxo)—vitamins A & D in oily solution for injection.
7-dehydrocholesterol—precursor of vitamin D_3.
dehydrogenase—an oxidising enzyme which acts by removing hydrogen from the substate.
dianol—" dimeride " of anol (*p*-hydroxypropenyl benzene).
Diœstrol (Frosst, Canada)—proprietary name for stilbœstrol.
dienœstrol—γ : δ-di-*p*-hydroxyphenyl-$\Delta^{\beta\delta}$-hexadiene or 4 : 4′-dihydroxy-γ : δ-diphenyl-β : δ-hexadiene, a synthetic œstrogen (B.D.H., Boots, Glaxo, Organon).
diethylstilbœstrol—synonymous with stilbœstrol.
dihydrotachysterol—A.T. 10 (*q.v.*).
Di-Menformon (Organon)—œstradiol benzoate.
Di-Ovocyclin (Ciba)—œstradiol dipropionate.
D.O.C.A. (Organon)—desoxycorticosterone (desoxycortone).
dysmenorrhœa—painful menstruation.

Emmenoplex (Glaxo)—œstriol.
endocrine—internally secreting, the secretion or hormone not being passed out through a duct.
endometrium—the decidual lining of the uterus.
Ephynal (Roche)—synthetic α-tocopherol acetate.
epimenorrhœa—excessively frequent menstruation.
equilenin } dehydrogenated steroids, excretion forms.
equilin } of œstradiol from equine urine.
ergosterol—sterol of the lower plants and precursor of calciferol (vitamin D_2).
Ertron (Nutrition Research Labs., Chicago)—" electrically activated " ergosterol, presumably pure calciferol.
Erugon (Bayer)—androsterone.
Erugon S (Bayer)—testosterone propionate.
Essogen (Lever Bros.)—vitamin A.

estrin	American spelling of	œstrin.
estrogen		œstrogen.
estradiol		œstradiol.
estrone		œstrone.
estriol		œstriol.

Estinyl (Schering, U.S.A.)—ethinyl œstradiol (*q.v.*).
ethinyl œstradiol—a synthetic, orally active œstrogen, the 17-acetylene derivative of œstradiol.
ethisterone—official name for pregneninolone (*q.v.*) (B.D.H., Boots)
exocrine—externally secreting, through a duct.
exophthalmia—protrusion of the eyeballs as in hyperthyroid states.

F.S.H.—follicle-stimulating hormone, serum gonadotropin.
Fallopian tubes—the tubes through which the ova pass between the ovary and uterine cavity.

Fertilol (Vits. Ltd.)—wheat germ oil concentrate (vitamin E).
flavoprotein—enzymes (dehydrogenases) containing riboflavine.
follicular hormone—œstradiol.
Follutein (Squibb)—chorionic gonadotropin.

Gestyl (Organon)—serum gonadotropin.
Glanduantin (Richter)—chorionic gonadotropin.
Glandubolin (Richter)—œstrone.
Glaxo—Glaxo Laboratories, Ltd., Greenford, Middlesex.
glossitis—soreness of the tongue, which is generally scarlet in nicotinamide-deficiency glossitis and magenta in riboflavine-deficiency glossitis.
glutathione—a tripeptide (of glutamic acid, cysteine and glycine), a coenzyme necessary for formation of lactic acid from glyceraldehyde.
glycogen—the storage form of the carbohydrates in animals, analogous to starch in plants, a " polymer " of glucose.
gonadotropin (or gonadotrophin)—anterior pituitary or chorionic gonad-stimulating hormone. Serum gonadotropin may be of pituitary origin.
gonadotropin—a gonad stimulating substance.
g., chorionic, luteinising gonadotropin secreted by the placenta and prepared from human pregnancy urine.
g., serum, follicule-stimulating gonadotropin from serum of pregnant mares.
Gonadyl (Roussel)—serum gonadotropin.
Gonan (B.D.H.)—chorionic gonadotropin.
gynœcogen—a substance producing female characteristics generally, *e.g.*, œstrogens and progestogens.
gynœcomastia—abnormal enlargement of the breasts in males.
Gynosone (I.C. Pharm.)—triphenylchloroethylene, a synthetic œstrogen.

hæmosiderosis—abnormal deposition of excess iron in body tissues in the form of an organic compound.
hæmatemesis—vomiting of blood.
herpes zoster—shingles.
hexœstrol—4 : 4′-dihydroxy-γ : δ-diphenyl-*n*-hexane, a synthetic œstrogen (B.D.H., B.W., Organon).
hormone—see p. 13 for definition.
Hykinone (Abbott)—2-methyl-1 : 4-naphthahydroquinone-3-sodium sulphonate, a synthetic analogue of vitamin K.
hypomenorrhœa—menstruation in which the flow is subnormal in amount and duration.
hypophamine (α- and β-)—posterior pituitary hormones (see p. 49).

ichthyosis—a skin disease in which the skin is scaly like that of a fish.
impotence—impotentia cœundi, failure of penile erection or male coital incompetence, sometimes wrongly used as meaning infertility, which is impotentia generandi.
I.C. Pharm.—I.C. (Pharmaceuticals), Ltd., 89 Oxford Street, Manchester, I.
Iletin (Lilly)—insulin.
inositol—hexahydroxy*cyclo*hexane, bios I, mouse anti-alopecia factor, a member of the vitamin B group.
insulin, delay (A. & H.), (B.D.H.)—insulin in solution which is precipitated immediately before use with a solution of protamine buffered with sodium phosphate.

insulin, globin (A. & H.) (B.D.H.) (Boots) (B.W.)—a solution of insulin and zinc sulphate with globin from hæmoglobin.
insulin, protamine, (with zinc) suspension (A. & H, B.D.H., Boots, B.W.)—a *suspension* of insulin with zinc sulphate and protamine.
iodogorgoic acid—3 : 5-diiodotyrosine.

Kappaxan (Bayer)—acetomenaphthone (tablets) and menaphthone (ampoules).
Ketodestrin (Paines & Byrne)—œstrone (ketohydroxyœstrin).
Kolpon (Organon)—œstrone (vaginal bougies and tablets).
kraurosis vulvæ—a condition of the vulva in which its mucosa becomes dry and glistening.
kryptoxanthine—a carotenoid pigment, precursor of vitamin A.

L.H.—luteinising hormone (gonadotropin), prolan B, or chorionic gonadotropin.
lactoflavine—a more or less obsolete name for riboflavine (vitamin B_2).
leucopenia—absence of leucocytes.
Luteoantin (Richter)—serum gonadotropin.
luteotropin—pituitary lactogenic hormone, probably identical with prolactin.
Lutogyl (Roussel)—progesterone.

menacme—the period between menarche and menopause (*q.v.*).
menarche—the onset of menstruation at puberty.
Menformon (Organon)—œstrone.
menopause—the cessation of menstruation (*cf.* climacteric).
methyl glyoxal—pyruvic aldehyde.
methyl-testosterone—orally active derivative of testosterone (B.D.H., Boots, B.W.)
methyl-thiouracil (B.D.H.), (Organon)—antithyroid substance for the treatment of hyperthyroidism.
metropathia hæmorrhagica—hæmorrhagic disease of the uterus.
myometrium—the muscular outer layer of the uterus.
myxoxanthine—a carotenoid pigment, precursor of vitamin A.

Nadola (P.D. & Co.)—vitamins A and D in oily solution.
Navitol (Squibb)—vitamins A and D in oily solution.
Neo-Hombreol—(Organon)—testosterone propionate.
Neo-Hombreol (M) (Organon)—methyl testosterone (*q.v.*).
Neo-œstranol I (Crookes)—stilbœstrol.
Neo-œstranol II (Crookes)—stilbœstrol dipropionate.

Octofollin—obsolete proprietary name for benzestrol (*q.v.*).
œstradiol—ovarian follicular hormone.
œstradiol benzoate—monobenzoic ester of œstradiol (B.W.)
œstradiol dipropionate—dipropionic ester of œstradiol.
œstrane—saturated hydrocarbon parent substance of the œstrogenic steroids.
œstrin—a general term for the natural œstrogens related to œstradiol.
œstriol—" trihydroxyœstrin," an excretion form of œstradiol.
Oestroform (B.D.H.)—natural œstrogenic hormones and their esters.
œstrogen—a substance, natural or synthetic, which produces the phenomena of œstrus in mammals or menstruation in primates (see gynœcogen).
Oestroglandol (Roche)—œstrone.

œstrone—ketohydroxyœstrin, $\Delta^{1,3,5}$-œstratriene-3-ol-17-one, a urinary œstrogen and partially inactivated œstradiol derivative.
œstrus—in mammals other than primates—heat, rut, the condition accompanying ovulation.
oligomenorrhœa—menstruation in which the volume of flow is subnormal (*cf.* hypomenorrhœa).
Oraluton (Schering)—ethisterone (*q.v.*).
Oraviron (Schering)—methyl testosterone.
Organon—Organon Laboratories, Ltd., Brettenham House, Lancaster Place, W.C.2.
Ostelin (Glaxo)—vitamin D_2.
Ovendosyn (Menley & James)—stilbœstrol with calcium phosphate.
Ovostab—œstradiol benzoate (Boots).
Ovocyclin-P (Ciba)—œstradiol dipropionate.

P.D. & Co.—Parke, Davis and Co., 50 Beak St., Regent St., W.1.
P.M.S.—pregnant mares' serum, serum gonadotropin.
Pabestrol (Paines & Byrne)—stilbœstrol.
Para-thor-mone (Lilly)—parathyroid extract.
phosphatase—an enzyme which catalyses transference of phosphorus from one compound to another.
phthiocol—2-methyl-3-hydroxy-1 : 4-naphthaquinone, pigment of mycobacterium tuberculosis, related to vitamin K.
phyone—growth hormone.
Physostab (Boots)—chorionic gonadotropin.
Phytoferol (B.D.H.)—α-tocopherol, capsules only.
picrocrocin—safranal glycoside.
Planavit C (M. & B.)—vitamin C.
polymenorrhœa—*vide* epimenorrhœa.
polypeptide—a substance built up of three or more amino acids but simpler than a protein.
pregnandiol—17-(α-hydroxyethyl)-ætiocholan)(α)-ol or pregnane-3(α)-20-diol, the inactive excretion form of progesterone.
pregneninolone—pregneninonol, anhydrohydroxyprogesterone, or ethisterone.
Pregnyl (Organon)—chorionic gonadotropin.
Prepalin (Glaxo)—a vitamin A concentrate.
progesterone—see progestin.
progestin (B.D.H., B.W., Organon)—progesterone, Δ^4-pregnene-3 : 20-dione.
progestogen—a hormone-producing progestational proliferation of the endometium.
Progestoral (Organon)—ethisterone.
Progynon (Schering)—œstradiol.
Progynon B Oleosum (Schering)—œstradiol benzoate.
Progynon D-P (Schering)—œstradiol dipropionate.
Prokayvit (B.D.H.)—menaphthone.
Prokayvit Oral (B.D.H.)—acetomenaphthone.
Prolan (Bayer)—chorionic gonadotropin.
prolan A—follicle-stimulating gonadotropin of the anterior pituitary.
prolan B—luteinising gonadotropin of the anterior pituitary.
Proluton (Schering)—progesterone.
Proluton C (Schering)—obsolete name for Oraluton (*q.v.*), ethisterone.
prosthetic group—a molecular substituent.
protamine—a group of simpler proteins from melt (sperm) of fishes.
Proviron (Schering)—androsterone.

pyridoxine (B.D.H., Vitamins, Ltd.)—vitamin B_6.
pyruvic aldehyde—methyl glyoxal.

Radiostol (B.D.H.)—vitamin D_2.
Radiostoleum (B.D.H.)—vitamins A and D in oily solution.
Redoxon (Roche)—vitamin C.
relaxin—a "nitrogenous" corpus luteum hormone—relaxes pelvic ligaments.
rhodeus amarus (Bloch)—the bitterling, a fish which has been used in pregnancy diagnosis tests.
Richter—Gedeon Richter (Great Britain), Ltd., Richter House, 14–18 Weedington Road, N.W.5.
riboflavine—issued under this name by B.D.H., B.W., Crookes.
Roche—Roche Products, Ltd., Broadwater Road, Welwyn Garden City, Herts., and 166 Buchanan Street, Glasgow, C.1.
Roussel—Roussel Laboratories, Ltd., 95 Great Portland Street, W.1.

Schering—British Schering, Ltd., 167–169 Great Portland Street, W.1.
Serogan (B.D.H.)—serum gonadotropin S.
Squibb—E. R. Squibb & Sons, c/o Savory & Moore, Ltd., Standard Works, Lawrence Road, Tottenham, N.15.
Sterandryl (Roussel)—testosterone propionate.
stilbœstrol (A. & H., Bayer, B.D.H., Boots, B.W., Organon.)—incorrectly known also as diethylstilbœstrol, 4 : 4′-di-hydroxy-α : β-diethyl stilbene.
stilbœstrol D.P. (B.D.H. Boots, B.W.)—stilbœstrol dipropionate.
Synapoidin (P.D. & Co.)—pituitary and chorionic gonadotropins.
synergistic factor—probably luteinising gonadotropin.
Synkamin (P.D. & Co.)—vitamin K_5, 4-amino-2-methyl-1-naphthol hydrochloride.
Synkavit (Roche)—vitamin K analogue, 2-methyl-1 : 4-naphtha-hydroquinone diphosphoric acid, tetrasodium salt.
Syntestrin (Richter)—stilbœstrol dipropionate.
Synthovo (Boots)—hexœstrol.

testosterone propionate—(Boots, B.D.H., B.W.).
Testoviron (Schering)—testosterone propionate.
Theelin (P.D. & Co.)—œstrone.
Theelol (P.D. & Co.)—œstriol.
Thelestrin (Carnrick)—œstrone.
threshold (renal)—the blood level of a substance above which the substance is excreted by the kidney.
Thyrogan (B.D.H.)—thyrotropin (discontinued).
thyrotropin—thyroid-stimulating hormone of anterior pituitary (*see also* Ambinon A & B and Thyrogan).
tocol—an alcohol, of which the 5 : 7 : 8-trimethyl derivative is α-tocopherol.
Tridestrin (Paines & Byrne)—œstriol.
trigonelline—nicotinic acid methyl betaine, an excretion form of nicotinic acid.
triphenylchloroethylene—a synthetic œstrogen.
trophin—a hormone which stimulates growth *and incidentally* secretion by another gland or secretory tissue.
tropin—a hormone which stimulates secretion or activity.

Unden (Bayer)—œstrone.

viosterol—irradiated ergosterol, impure vitamin D_2.
vitamin—see page 146 for definition.
vitamin A—issued under this name by Crookes (capsules only).
vitamin B_1—issued under this name by A & H, B.D.H., B.W., Crookes, Paines & Byrne.
vitamin B_2—riboflavine (*q.v.*).
vitamin B_2 complex—more correctly, vitamin B_2 group—the whole of the vitamin B group excluding vitamin B_1. See Index and appropriate chapters for individual members.
vitamin B_6—pyridoxine.
vitamin B_6 group—pyridoxine, pyridoxal, pyridoxamine.
vitamin B_7—obsolete designation for nicotinamide.
vitamin B_C—folic acid.

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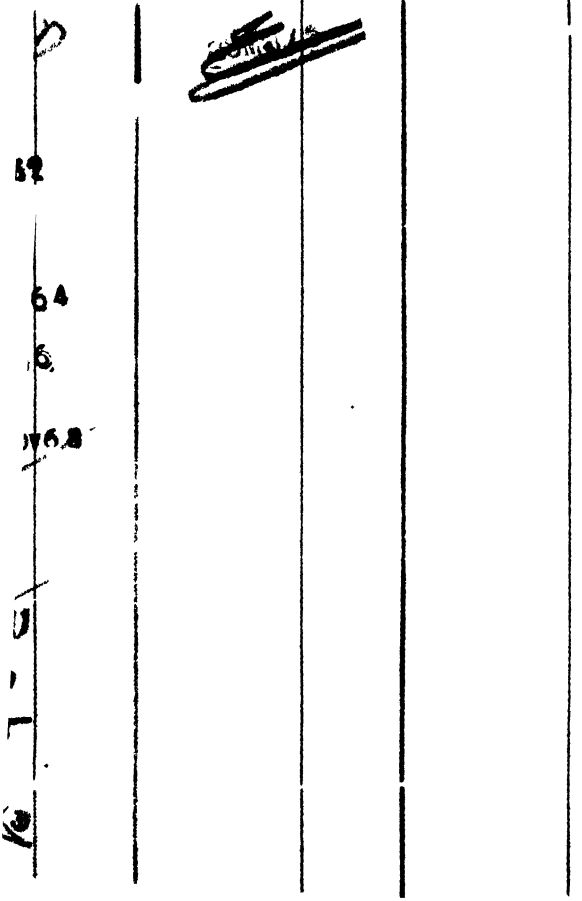
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